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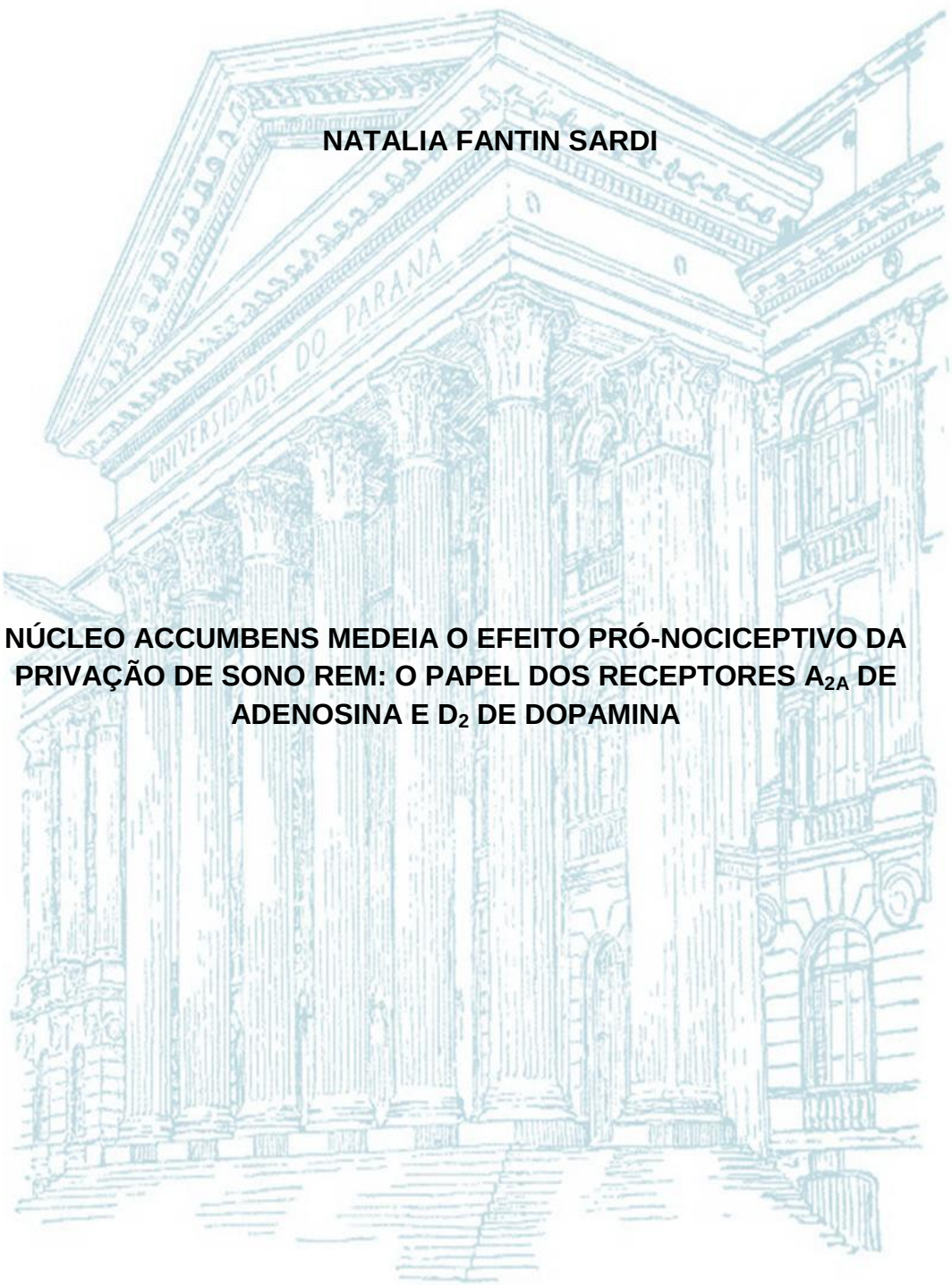
NATALIA FANTIN SARDI



NÚCLEO ACCUMBENS MEDEIA O EFEITO PRÓ-NOCICEPTIVO DA PRIVAÇÃO DE SONO REM: O PAPEL DOS RECEPTORES A_{2A} DE ADENOSINA E D_2 DE DOPAMINA

CURITIBA

2017

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Dissertação apresentada ao Curso de Pós-Graduação em Fisiologia, Setor de Ciências Biológicas, da Universidade Federal do Paraná, como requisito parcial à obtenção do grau de Mestre em Fisiologia.

Orientadora: Profª. Dra. Luana Fischer.

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Setor de Ciências Biológicas
Departamento de Fisiologia
Programa de Pós-Graduação em Fisiologia

UFPR Biológicas

Ata da defesa de dissertação de mestrado de NATÁLIA FANTIN SARDI

Aos treze dias do mês de fevereiro do ano de dois mil e dezessete, foi realizada no auditório do Departamento de Fisiologia no Setor de Ciências Biológicas da Universidade Federal do Paraná, a defesa de dissertação da mestranda **NATÁLIA FANTIN SARDI,** intitulada **"NÚCLEO ACCUMBENS MEDEIA O EFEITO PRÓ-NOCICEPTIVO DA PRIVAÇÃO DE SONO REM: O PAPEL DOS RECEPTORES A_{2A} DE ADENOSINA E D_2 DE DOPAMINA".** A abertura teve início às 13h30min pela Presidente da Banca Examinadora e Orientadora da candidata, Professora Doutora Luana Fischer. A Presidente apresentou ao público presente os membros da banca examinadora e logo passou a palavra à aluna, para que fizesse uma apresentação sucinta de sua dissertação. Após a explanação oral, a Professora Doutora Luana Fischer passou à palavra ao primeiro examinador, Professora Doutora Cláudia Herrera Tambeli do Departamento de Biologia Estrutural e Funcional da UNICAMP. Na sequência, passou a palavra ao segundo examinador, Professor Doutor Bruno Jacson Martynhak do Departamento de Fisiologia da UFPR. A aluna respondeu as perguntas dos examinadores e se posicionou frente às críticas. Findas as arguições pelos demais membros da banca, a Presidente, Professora Doutora Luana Fischer fez uma rápida apreciação das conclusões mais importantes dos debates realizados e comunicou que a Banca Examinadora iria reunir-se em sessão secreta para discussão e atribuição dos conceitos. Os trabalhos foram interrompidos por cinco minutos. Após haver analisado o referido trabalho e argüido a candidata, os membros da banca examinadora reunidos em sessão secreta deliberaram pela "APROVAÇÃO", habilitando-a ao título de Mestre em Fisiologia, condicionada à implementação das correções sugeridas pelos membros da banca examinadora e ao cumprimento integral das exigências estabelecidas no Art. 59º do Regimento interno deste Programa de Pós-Graduação. Eu, Professora Doutora Luana Fischer, Presidente da Banca Examinadora lavrei a presente ata, da qual assino juntamente com os senhores examinadores.

Curitiba, 13 de fevereiro de dois mil e dezessete.

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Sou feita de retalhos.

Pedacinhos coloridos de cada vida que passa pela minha e que vou costurando na alma.

Nem sempre bonitos, nem sempre felizes, mas me acrescentam e me fazem ser quem eu sou.

Em cada encontro, em cada contato, vou ficando maior...

Em cada retalho, uma vida, uma lição, um carinho, uma saudade...

Que me tornam mais pessoa, mais humana, mais completa.

E penso que é assim mesmo que a vida se faz: de pedaços de outras gentes que vão se tornando parte da gente também.

E a melhor parte é que nunca estaremos prontos, finalizados...

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Sou feita de retalhos – Cora Coralina

RESUMO

Distúrbios de sono alteram a sensibilidade à dor e predispõem ao desenvolvimento de condições dolorosas. No entanto pouco é conhecido a respeito dos mecanismos pelos quais a privação de sono afeta a dor. O Núcleo Accumbens (NAc), no estriado ventral, desempenha importante papel na modulação da dor e na regulação do ciclo sono/ vigília. No entanto, não se sabe se este núcleo medeia o efeito pró-nociceptivo da privação de sono. Desse modo, o objetivo deste trabalho foi testar a hipótese de que o NAc medeia o efeito pró-nociceptivo da privação se sono REM (movimento rápido dos olhos) e, caso medeie, investigar os mecanismos envolvidos. A privação de sono REM por 24 horas através do método de plataforma única induziu um efeito pró-nociceptivo intenso e duradouro, demonstrado pela diminuição do limiar nociceptivo mecânico em ratos Wistar. Esse efeito diminui progressivamente ao longo do período de sono rebote, mas ainda permanece significativo 48 h depois. A atividade motora, aferida por actimetria, é significativamente diminuída na fase escura em ratos privados de sono, o que é compatível com um aumento do tempo de sono após a privação. A lesão excitotóxica induzida por N-metil D-Aspártico (NMDA) no NAc preveniu o efeito pró-nociceptivo da privação de sono REM enquanto o bloqueio agudo do NAc por Qx-314 (2%), um derivado quaternário de lidocaína, reverteu esse efeito. Esses dados mostram que o NAc medeia o efeito pró-nociceptivo da privação de sono REM, sendo essencial à sua indução e manutenção. Uma vez que o NAc regula o ciclo sono-vigília através de um balanço entre a atividade adenosinérgica sobre receptores A_{2A} e a atividade dopaminérgica sobre receptores D_2, investigamos se estes receptores também medeiam o efeito pró-nociceptivo da privação de sono REM. A administração de um antagonista do receptor A_{2A} (SCH 58261, 7 ng) ou de um agonista do receptor D_2 (Piribedil, 6 µg) no NAc aumentou a atividade dos animais e bloqueou o efeito pró-nociceptivo da privação de sono REM. De forma complementar, a administração de um agonista do receptor A_{2A} (CGS 21680, 24 ng) ou de um antagonista do receptor D_2 (Raclopride, 5 µg) no NAc diminuiu a atividade e pelo menos o agonista do receptor A_{2A} prejudicou a reversão do efeito pró-nociceptivo durante o período de sono rebote. A privação de sono REM não afetou a expressão da proteína c-fos no NAc. Juntos os dados obtidos no presente trabalho sugerem que privação de sono REM aumenta a dor por aumentar a atividade adenosinérgica sobre receptores A_{2A} e diminuir a atividade dopaminérgica sobre receptores D_2 localizados no NAc. Entender os mecanismos pelos quais prejuízos no sono aumentam a dor é essencial para que se obtenha sucesso no complexo manejo da dor em pacientes que sofrem de distúrbios de sono.

Palavras-chave: nocicepção; Dor; Núcleo Accumbens; Privação de sono REM; Adenosina; Dopamina.

ABSTRACT

Sleep disorders alter pain sensitivity and predispose the development of painful conditions. However little is known about the mechanisms by which sleep deprivation affects pain. The Nucleus Accumbens (NAc), in the ventral striatum, plays an important role in pain modulation and in sleep-wake cycle regulation. However, it is not known whether NAc mediates the pronociceptive effect of sleep deprivation. Thus, the objective of this study was to test the hypothesis that the NAc mediates the pronociceptive effect of REM (rapid eye movement) sleep deprivation and, if it mediates, investigate the underline mechanisms. A 24 hours REM sleep deprivation through the single platform method induced an intense and long-lasting pronociceptive effect, demonstrated by the decrease of mechanical nociceptive threshold in Wistar rats. This effect decreases progressively over the rebound sleep period, but still remains significant 48 h later. The activity, measured by actimetry, was significantly decreased in the dark phase in sleep deprived rats, which is compatible with an increase in sleep time after deprivation. N-methyl D-Aspartic (NMDA) induced excitotoxic lesion in NAc, prevented the pronociceptive effect of REM sleep deprivation while the acute blockade by Qx-314 (2%), a quaternary derivative of lidocaine, reversed this effect. These data show that NAc mediates the pronociceptive effect of REM sleep deprivation, being essential to its induction and maintenance. Since NAc regulates the sleep-wake cycle through a balance between adenosine activity on A_{2A} receptors and dopaminergic activity on D_2 receptors, we investigated whether these receptors also mediate the pronociceptive effect of REM sleep deprivation. Administration of an A_{2A} receptor antagonist (SCH 58261, 7 ng) or a D_2 receptor agonist (Piribedil, 6 µg) in NAc increased animal activity and blocked the pronociceptive effect of REM sleep deprivation. Complementarily, administration of an A_{2A} receptor agonist (CGS 21680, 24 ng) or a D_2 receptor antagonist (Raclopride, 5 µg) in NAc decreased activity and at least the A_{2A} receptor agonist impaired the reversal of the pronociceptive effect during the sleep rebound period. REM sleep deprivation did not affect expression of c-fos protein in NAc. Together the data obtained in the present study suggest that REM sleep deprivation increases pain by increasing NAc adenosinergic A_{2A} activity and by decreasing NAc dopaminergic D_2 activity. The understanding of the mechanisms by which sleep loss affect nociception will contribute to pain management in patients suffering from sleep disorders.

Keywords: Nociception; Pain; Nucleus Accumbens; REM sleep deprivation; Adenosine; Dopamine.

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LISTA DE ABREVIAÇÕES

NAc – Núcleo Accumbens

REM – movimento rápido dos olhos do inglês “*Rapid eye moviment*”

PS REM – Privação de sono REM

PS – Privação de sono

NREM – Sono não REM

PAG – Substância Cinzenta Periaquedutal do inglês “*Periaqueductal Gray Matter*”

RVM – Bulbo rostro ventral do inglês “*Rostral ventral medulla*”

VTA – Área Tegmental ventral do inglês “*Ventral tegmental area*”

NMDA – ácido N-Metil D-Aspártico

REM SD – REM sleep deprivation

SD – sleep deprivation

EEG – Eletroencefalograma

A_{2A} – receptores de adenosina do tipo A_{2A}

D_2 – receptores de dopamina do tipo D_2

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1. INTRODUÇÃO

As condições persistentes de dor e os distúrbios de sono podem ser consideradas problemas de saúde pública em todo o mundo (Chiu et al., 2005, Ohayon, 2005, Artner et al., 2013, Kim et al., 2015) e o vínculo bidirecional entre eles está bem estabelecido (Morin et al., 1998, Nicholson and Verma, 2004, Smith and Haythornthwaite, 2004, Okifuji and Hare, 2011, Karaman et al., 2014). Por exemplo, os distúrbios de sono estão presentes em até 88% dos pacientes com dor crônica (Smith and Haythornthwaite, 2004, Morin et al., 2006, Cheatle et al., 2016) e pelo menos 50% dos indivíduos com insônia sofrem de dor crônica (Taylor et al., 2007). No entanto, embora não haja dúvidas de que a diminuição de sono aumenta a dor em seres humanos (Lentz et al., 1999, Arima et al., 2001, Onen et al., 2001b, Smith and Haythornthwaite, 2004) e diminua o limiar nociceptivo em animais (Onen et al., 2000, Onen et al., 2001a, Onen et al., 2001b, Nascimento et al., 2007, Wei et al., 2007, Wei et al., 2008, Damasceno et al., 2009, Wei et al., 2010, Skinner et al., 2011) os mecanismos são em grande parte desconhecidos.

Recentemente, demonstrou-se que a privação de sono REM (movimento ocular rápido) aumenta as respostas nociceptivas em ratos, por facilitar a transmissão da informação nociceptiva no sistema descendente PAG-RVM (substância cinzenta periaquedutal - bulbo rostro ventral) (Tomim et al., 2016), o mecanismo de modulação da dor mais estudado (Millan, 2002). As vias descendentes PAG-RVM servem como um sistema de saída que integra aferências de várias regiões do prosencéfalo para modular a transmissão nociceptiva no corno dorsal espinhal (Millan, 2002). Estudos sugerem a participação da formação reticular pontina na modulação da dor (Watson et al., 2010, 2014) e no efeito pró-nociceptivo da privação de sono (Vanini et al., 2014). No entanto, se outras regiões do prosencéfalo envolvidas na modulação endógena da dor também estão envolvidas no efeito pró-nociceptivo da privação de sono não é conhecido.

O núcleo accumbens (NAc), localizado no estriado ventral, é um dos núcleos do prosencéfalo envolvidos na modulação da dor (Gear and Levine, 2011, Tobaldini et al., 2014) e também no ciclo sono-vigília (Lazarus et al., 2013). O papel do NAc no controle do ciclo sono-vigília foi demonstrado por vários estudos, por exemplo, sua lesão neurotóxica aumenta a vigília (Monti et al., 1999, Qiu et al., 2010, Qiu et al.,

2012). Recentemente, foi sugerido que o NAc induz o sono inibindo, através de projeções GABAérgicas, núcleos promotores de vigília no tronco encefálico e hipotálamo (Lazarus et al., 2013). Supõe-se que esta projeção inibitória do NAc seja dependente do equilíbrio entre a dopamina que atua sobre os receptores inibitórios D_2 para induzir a vigília e a adenosina que atua sobre os receptores A_{2A} excitatórios para induzir sono. Por exemplo, a ativação dos receptores A_{2A} no NAc induz o sono (Satoh et al., 1999), enquanto a sua deleção seletiva no NAc evita a vigília induzida pela cafeína (Lazarus et al., 2011). Complementarmente, quando administrados agonistas D_2 no NAc, o sono diminui, enquanto os antagonistas aumentam o sono (Barik and de Beaurepaire, 2005, Monti and Jantos, 2008, Qu et al., 2010). O papel do NAc na modulação da dor também tem sido amplamente estudado. Embora nenhum estudo prévio tenha abordado o envolvimento dos receptores A_{2A}, a ativação dos receptores D_2 no NAc está associada ao alívio da dor (Altier and Stewart, 1998, 1999, Taylor et al., 2003, Haghparast et al., 2012). Portanto, é possível que o NAc contribua para o efeito pró-nociceptivo da privação de sono REM, e se assim for, é possível que essa contribuição dependa de uma atividade adenosinérgica aumentada nos receptores A_{2A} e de uma diminuição da atividade dopaminérgica nos receptores D_2.

Portanto, o primeiro objetivo deste estudo foi determinar se o NAc contribui para o efeito pró-nociceptivo da privação de sono REM. O segundo objetivo era determinar o envolvimento dos receptores A_{2A} de adenosina no NAc e/ou dos receptores D_2 de dopamina neste efeito. O NAc foi bloqueado agudamente, previamente lesionado ou micro infundido com agonistas e antagonistas seletivos dos receptores A_{2A} ou D_2. A resposta nociceptiva e a atividade geral foram determinadas antes e imediatamente após um período de 24 horas de privação de sono REM e, posteriormente, ao longo de um período de 48 horas de recuperação de sono. A expressão de c-Fos no NAc foi determinada e utilizada como um marcador indireto de atividade neuronal.

2. REVISÃO DE LITERATURA

2.1 SONO

O sono é uma das mais misteriosas funções fisiológicas do cérebro. Sono ou estados semelhantes a ele estão presentes em todos os organismos complexos que possuem um sistema nervoso central. O sono é comumente dividido em dois estágios, o sono REM (do inglês rapid eye movement) e sono não REM (NREM) classificados através de exames como eletroencefalograma (EEG) e o eletromiograma (Brown et al., 2012). O sono NREM é caracterizado por ondas de baixa frequência e grande amplitude, com atividade muscular diminuída. O sono REM, à semelhança da vigília, apresenta ondas de baixa voltagem e alta frequência, mas está associado a atonia muscular e a movimentos rápidos dos olhos (Brown et al., 2012).

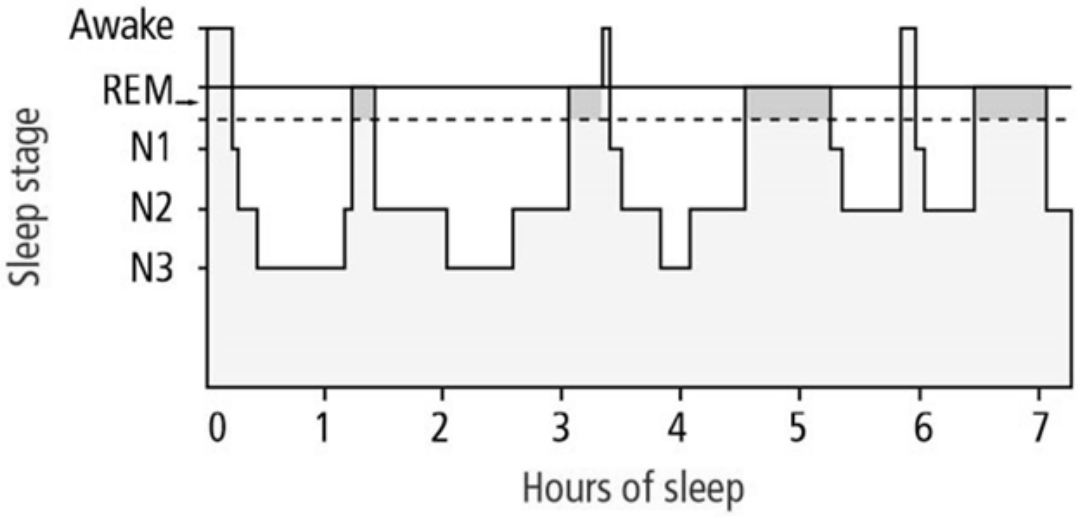

FIGURA A - Arquitetura de sono. Representação de um hipnograma que mostra a noite de sono típica de um adulto jovem e saudável. Observamos que a maior parte das horas de sono se concentra nos estágios de sono NREM (N1, N2 e N3) e um período menor de tempo se concentra no estágio REM (Figura adaptada de: Um guia para uma boa noite de descanso, um relatório especial de saúde publicado pela Harvard Health Publications. Disponível em: http://www.helpguide.org/harvard/biology-of-sleep-circadian-rhythms-sleep-stages.htm#nonREM).

O primeiro marco histórico referente a estudos de sono teve início em 1929, com a obtenção de registros de EEG em humanos, na qual o primeiro registro das ondas cerebrais foi obtido pelo psiquiatra alemão Hans (Berger, 1929). Após essa descoberta, Loomis e coautores, foram os primeiros a diferenciar as fases da vigília,

sono e sonhos no EEG em humanos (Loomis et al., 1935). Por meio de suas pesquisas, eles identificaram os estágios de sono e vigília, como também a fase em que os sonhos ocorrem e que esta se encontra com ondas cerebrais em baixa amplitude. Logo após, o cientista alemão Rudolf Klaue, começou a estudar o EEG em gatos, e descobriu uma sequência característica de sono, em que ocorre um período de sono leve, em que o córtex produz ondas lentas, seguido por um período de sono mais profundo, ocorrendo um aumento da atividade cortical (Klaue, 1937). Essa fase de sono profundo, só foi denominada de paradoxal, após pesquisas de Aserinsky e Kleitman, que associaram essa fase com movimento ocular rápido (Aserinsky and Kleitman, 1953).

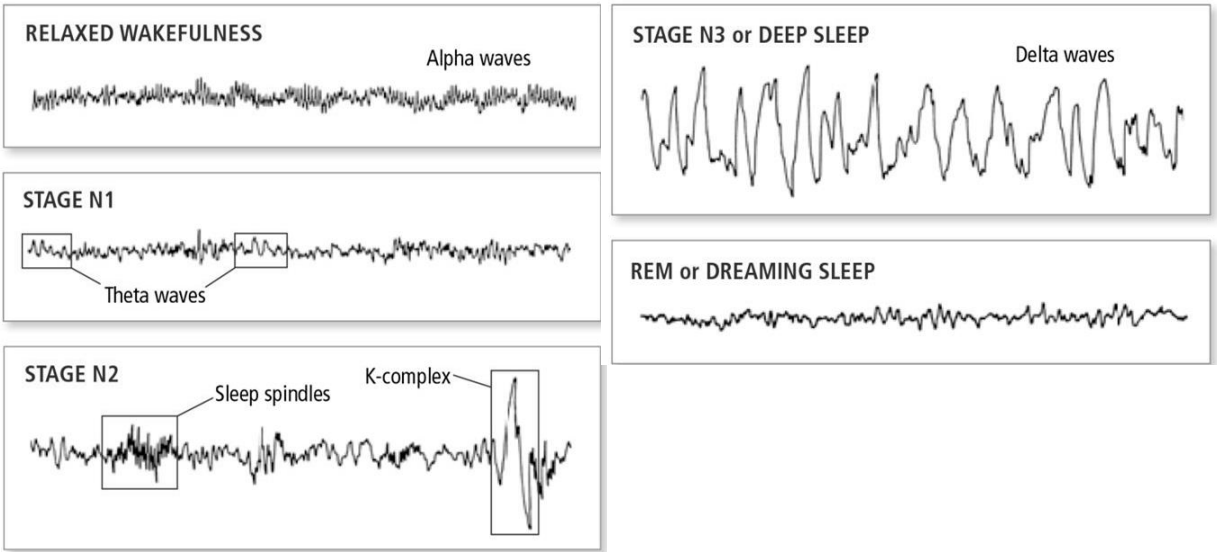

FIGURA B – Representação de um EEG demonstrando que as ondas cerebrais mudam dramaticamente durante as diferentes fases de sono (Figura adaptada de: Um guia para uma boa noite de descanso, um relatório especial de saúde publicado pela Harvard Health Publications. disponível em: http://www.helpguide.org/harvard/biology-of-sleep-circadian-rhythms-sleep-stages.htm#nonREM).

Uma das funções de sono consiste em manter o organismo em estado de repouso para desempenhar as funções no restabelecimento e manutenção da homeostasia orgânica, que ocorrem principalmente enquanto o indivíduo está dormindo (Andersen et al., 2004, Nascimento et al., 2007, Son et al., 2011).

Os hábitos de sono dos seres humanos, são únicos entre os animais no sentido que, muitas vezes desafiamos o sono e ficamos acordados por razões profissionais ou de lazer, embora estejamos cansados. A motivação para permanecer acordado e ativo na sociedade moderna é cada vez maior e isso tem sido associado ao

significativo aumento na prevalência de distúrbios de sono (Owens, 2014). De fato, estudos baseados nos dados do EPISONO – pesquisa epidemiológica realizada pelo Instituto de sono na cidade de São Paulo – revelam que cerca de 77% da população da cidade de São Paulo sofre com algum distúrbio relacionado ao sono (Santos-Silva et al., 2009, Andersen et al., 2010, Tufik et al., 2010, Cintra et al., 2012, Santos-Silva et al., 2012, Polese et al., 2013). Tais distúrbios geram grande impacto social e econômico, pois estão associados a condições psiquiátricas, acidentes por prejuízo na atenção, diminuição de produtividade (Brown et al., 2012), aumento da prevalência e severidade de condições dolorosas (Morin et al., 1998, Smith et al., 2000, Nicholson and Verma, 2004) entre outros problemas. Embora os distúrbios de sono possam ocorrer em qualquer fase de sono (Brown et al., 2012), a maioria destes distúrbios se caracteriza por prejuízos ou diminuição no REM, que ocorre, em geral, durante a segunda metade da noite (Carli et al., 1987). Dentre os distúrbios podemos citar, narcolepsia, desordem comportamental de sono REM, síndrome de pernas inquietas, movimentos periódicos de pernas e desordens de estresse pós traumático (Brown et al., 2012). Por esta razão, a maioria dos estudos experimentais se dedica a estudar os efeitos associados à diminuição de sono REM.

2.2 RELAÇÃO ENTRE SONO E DOR

Assim como os distúrbios de sono, a dor tem se tornado um problema cada vez mais comum na sociedade moderna. Não há dados epidemiológicos sobre a incidência e prevalência de condições dolorosas persistentes na população brasileira em geral. Mas se extrapolarmos dados da população norte americana (I.O.M, 2011) para a brasileira, chegaremos ao número de setenta e três milhões de pessoas sofrendo com algum tipo de dor persistente no país. Certamente este é um dos maiores problemas da saúde pública, possivelmente mundial, que além de afetar diretamente a qualidade de vida de milhões de pessoas, está associado a um gigantesco impacto econômico. Só nos Estados Unidos, a estimativa de custo com tratamento e perda de produtividades relacionadas à dor apenas no ano de 2010 foi estimada em pelo menos 635 bilhões de dólares (Gaskin and Richard, 2012).

Recentemente vários estudos têm investigado a relação entre dor e distúrbios de sono (Onen et al., 2001a, Onen et al., 2001b, Andersen et al., 2004, Nascimento et al., 2007, Wei et al., 2007, Wei et al., 2008, Damasceno et al., 2009, Skinner et al.,

2011, Tomim et al., 2016). A partir desses estudos ficou claro que essa é uma relação bidirecional, uma vez que a dor pode causar distúrbios de sono (Nicholson and Verma, 2004, Okifuji and Hare, 2011) e alterações do padrão normal de sono podem predispor ao desenvolvimento de condições dolorosas (Morin et al., 1998, Nicholson and Verma, 2004, Smith and Haythornthwaite, 2004, Karaman et al., 2014), aumentar a sensibilidade à dor em humanos (Lentz et al., 1999, Arima et al., 2001, Onen et al., 2001b, Smith and Haythornthwaite, 2004) e diminuir o limiar nociceptivo em animais (Onen et al., 2000, Onen et al., 2001a, Onen et al., 2001b, Nascimento et al., 2007, Wei et al., 2007, Wei et al., 2008, Damasceno et al., 2009, Wei et al., 2010, Skinner et al., 2011, Damasceno et al., 2013).

O crescente número de estudos investigando a interação entre sono e dor e a unanimidade de resultados indicando que a privação de sono (PS) tem papel pró-nociceptivo contrasta com o pobre entendimento a respeito dos mecanismos pelos quais a PS aumenta a dor. A maioria dos estudos que investigou tais mecanismos se concentrou em mecanismos espinhais, ou utilizaram intervenções sistêmicas. Por exemplo, já foi demonstrado que a PS REM aumenta a atividade glutamatérgica (Wei et al., 2007), aumenta a atividade serotonérgica (Wei et al., 2008) e diminui a atividade GABAérgica (Wei et al., 2010) na medula espinhal. Também foi demonstrado que o efeito antinociceptivo induzido pela administração sistêmica de agonistas opioides (Nascimento et al., 2007, Skinner et al., 2011) ou agonista dopaminérgico é diminuído pela PS REM (Skinner et al., 2011). Um estudo recente de nosso laboratório contribuiu para melhorar o entendimento a respeito dos mecanismos pelos quais a PS REM aumenta a dor. Este estudo demonstrou que o potente efeito pró-nociceptivo da PS está associado à diminuição da ativação das vias descendentes de inibição da dor e aumento da ativação das vias descendentes de facilitação da dor (Tomim et al., 2016). Esta é a primeira evidência de que a PS aumenta a dor por modular o mais conhecido e potente sistema endógeno de modulação da dor, o sistema descendente. O centro de tal sistema é a PAG (do inglês, *Periaqueductal Gray Matter*), que recebe múltiplas aferências de diferentes regiões encefálicas e as integra para controlar indiretamente, através de vias descendentes que partem do RVM (do inglês, *Rostal Ventral Medulla*), a transmissão da informação nociceptiva na medula espinhal (Millan, 2002, Fields, 2004).

2.3 DOR E NÚCLEO ACCUMBENS

Além da PAG e do RVM outros núcleos encefálicos apresentam papel fundamental no controle endógeno da dor. Entre eles o Núcleo Accumbens (NAc) se destaca, pois além de seu papel na modulação da dor (Gear and Levine, 2011) ele controla importantes aspectos da locomoção, comportamento motivacional, sistema de recompensa e ciclo sono - vigília (Lazarus et al., 2013). Localizado no estriado ventral, o NAc exerce suas complexas funções ao integrar a informação proveniente principalmente de projeções do hipocampo (informação contextual), da amígdala (conteúdo emocional), do córtex pré-frontal, (informação cognitiva) e da área tegmental ventral (VTA, do inglês Ventral Tegmental Area, centro do sistema dopaminérgico mesocorticolímbico) (Heimer et al., 1991, Lu et al., 1998, Groenewegen et al., 1999). Funcionalmente dividido em duas regiões, shell e core, admite-se que as eferências do NAc sejam em sua imensa maioria GABAérgicas e se dividam em duas vias que projetam, em última instância, para a Substância Negra. A via estriado nigral ou direta, cujos neurônicos expressam também substância P e receptores para acetilcolina M_4, e dopamina D_1; e a via estriado palidal, ou indireta, cujos neurônicos expressam também encefalina e receptores para dopamina D_2 e adenosina A_{2A} (Durieux et al., 2011).

Evidências recentes demonstram o papel do NAc na nocicepção e sugerem que as eferências deste núcleo sejam pró-nociceptivas, uma vez que a injeção intra-NAc de kainato, agonista de receptores de aminoácidos excitatórios, produz efeito pró-nociceptivo enquanto a injeção de agonistas opioides ou de anestésico intra-NAc produz efeito antinociceptivo (Schmidt et al., 2002b, Gear and Levine, 2011). Além disso, um aumento da atividade mesolímbica dopaminérgica promove analgesia (Altier and Stewart, 1998, 1999, Magnusson and Fisher, 2000, Haghparast et al., 2012), enquanto a diminuição desta atividade induz hiperalgesia (Saade et al., 1997, Magnusson and Martin, 2002). De fato, a ativação do receptor D_2 (acoplado a proteína G inibitória) no NAc está associada a analgesia em modelos de dor aguda enquanto a participação do receptor D_1 (acoplado a proteína G estimulatória) não está muito clara (Altier and Stewart, 1998, 1999, Schmidt et al., 2002a, Taylor et al., 2003, Haghparast et al., 2012, Dias et al., 2015), mas que cerca de 95% da população de neurônios do NAc expressa receptores do tipo D_1 e D_2 (Hasbi et al., 2009, Perreault et al., 2011).

2.4 NÚCLEO ACCUMBENS E SONO

Outros alvos das projeções do NAc incluem o pálido ventral, o hipotálamo lateral, VTA e o núcleo parabraquial (Lazarus et al., 2012, Lazarus et al., 2013). O núcleo parabraquial é a maior fonte de entrada do tronco cerebral para o prosencéfalo basal e também possui projeções substanciais para o hipotálamo lateral (Lazarus et al., 2012, Lazarus et al., 2013). Assim sendo, o núcleo parabraquial é caracterizado como um componente chave do sistema de ativação ascendente, responsável pela ativação cortical e consequente estado de vigília, de fato, lesões no núcleo parabraquial causam coma (Fuller et al., 2011). Desta maneira, a ativação do NAc exerceria efeitos inibitórios sobre esses importantes sítios indutores de vigília promovendo assim, o sono e contribuindo para a regulação do ciclo sono/vigília (Lazarus et al., 2012, Lazarus et al., 2013).

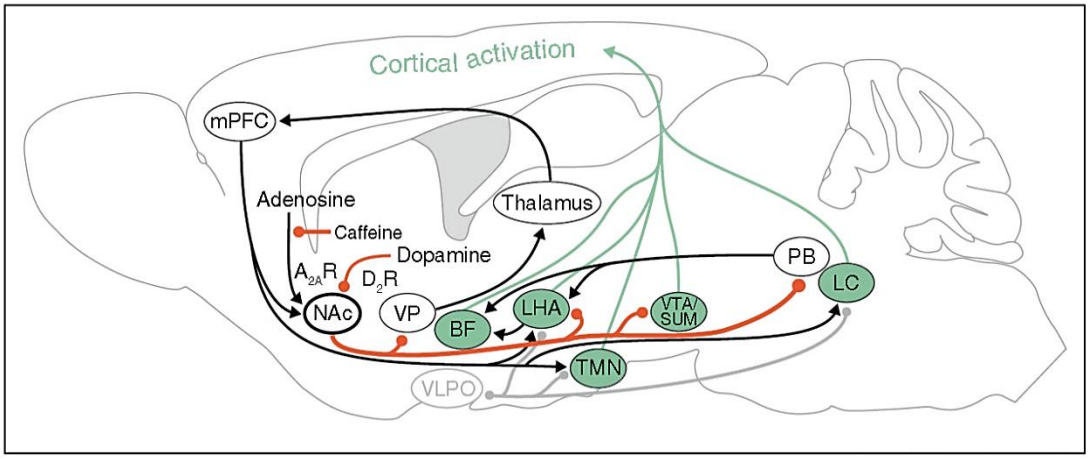

Figura C – Modelo no qual o núcleo accumbens (NAc) tem um papel intrínseco na rede reguladora do ciclo sono-vigília. As projeções de saída inibitórias do NAc modulam a atividade das populações neuronais no pálido ventral, no hipotálamo lateral (LHA), no núcleo parabraquial (PB) e na área tegmental ventral (VTA), que podem ser fontes mais ou menos importantes de excitação. O NAc pode modular o córtex pré-frontal medial (mPFC) através de uma via através do pálido ventral e do tálamo; E, por sua vez, o mPFC projeta neurônios promotores de excitação no núcleo tuberomamilar hipotalâmico (TMN), LHA e locus coeruleus (LC). O NAc auxilia os neurônios orexinérgicos e glutamatérgicos no LHA a enviar projeções principalmente para o prosencéfalo basal (BF) e córtex cerebral. O LHA também está conectado reciprocamente com áreas de "flip-flop" entre o sono não REM e a vigília (mostrada em prata), incluindo a área pré-óptica ventrolateral (VLPO), o TMN e o LC; E o PB, um componente importante do sistema de excitação ascendente, é conhecido por estar fortemente ligado ao BF e ao LHA. Os neurônios glutamatérgicos na borda entre a VTA e o núcleo supramamilar (SUM) também podem retransmitir o estímulo de vigília do NAc para o córtex cerebral. A adenosina que atua sobre os receptores A_{2A} excitatórios (A_{2A}Rs), em oposição ao sistema inibitório de dopamina/receptor D_2 (D_2R), pode modular a atividade dos neurônios GABAérgicos de saída no NAc para inibir a excitação e promover o sono. Setas pretas, excitação; Linhas vermelhas de extremidade redonda, inibição; As linhas pratas com duas extremidades redondas representam

ligações inibitórias recíprocas; Áreas circundadas com fundo verde, populações neuronais com projeções corticais (setas verdes claras) Adaptado de Lazarus (2013).

De fato, a lesão induzida no NAc por ácido ibotênico (Qiu et al., 2010, Qiu et al., 2012) ou por 6-hidroxidopamina (Monti et al., 1999), toxina que destrói seletivamente terminais dopaminérgicos, aumenta o tempo de vigília. Os neurônios GABAérgicos do NAc que expressam receptores D_2 para dopamina parecem ser os mais importantes para a regulação de sono, uma vez que a administração local de agonista D_2 diminui e de antagonista D_2 aumenta o tempo de sono (Barik and de Beaurepaire, 2005, Monti and Jantos, 2008, Qu et al., 2010). Esses são os mesmos neurônios que expressam receptores A_{2A} de adenosina (Ferre et al., 2007, Zhang et al., 2013). De fato, o estriado ventral é a região encefálica com maior densidade de receptores para dopamina e adenosina (Jarvis and Williams, 1989, Schiffmann et al., 1991, Lillrank et al., 1999). A adenosina é um importante fator homeostático indutor de sono, seu acúmulo no encéfalo durante a vigília induz sono (Porkka-Heiskanen et al., 1997, Porkka-Heiskanen et al., 2000, Porkka-Heiskanen et al., 2002, Huang et al., 2005). Diversos estudos tem sugerido importância fundamental dos receptores A_{2A} de adenosina localizados no NAc, especialmente na região shell, na indução de sono. Por exemplo, nessa região agonistas de receptores A_{2A} promovem sono (Satoh et al., 1999) enquanto o bloqueio na expressão (knockdown) desses receptores abole os efeitos estimulantes da cafeína (Lazarus et al., 2011), um conhecido antagonista A_{2A}. Estas observações levaram a ideia de que os receptores A_{2A} de adenosina, e D_2 de dopamina interagem para controlar as eferências GABAérgicas do NAc que inibem importantes centros promotores da vigília (Lazarus et al., 2012, Lazarus et al., 2013). Além de interação funcional, que se dá por estarem expressos nos mesmos neurônios, estes receptores também interagem de forma alostérica, formando heterodímero (Ferre, 1997, Ferre et al., 1997, Ferre et al., 2015). Embora o papel desses heterodímeros na regulação do ciclo sono vigília não tenha sido estudado, sabe-se que a ativação dos receptores D_2 de dopamina inibe a adenilato ciclase, inibindo as projeções GABAérgicas do NAc, enquanto a ativação de receptores A_{2A} ativa a adenilato ciclase, ativando tais projeções. A indução de sono seria fortemente influenciada, portanto, pelo balanço entre os níveis endógenos de adenosina e dopamina no NAc.

3. JUSTIFICATIVA E OBJETIVOS

Apesar de todas as evidências a respeito do importante papel do NAc na modulação da dor e na regulação do ciclo sono-vigília, a participação desse núcleo no efeito pró-nociceptivo da PS nunca foi estudado. Portanto, o primeiro objetivo deste projeto foi testar a hipótese de que o NAc contribui para o efeito pró-nociceptivo da PS REM. Dados preliminares, obtidos em nosso laboratório, sugeriam que esta hipótese seria confirmada, e, neste caso, estudamos o envolvimento de receptores A_{2A} de adenosina e D_2 de dopamina localizados no NAc no efeito pró-nociceptivo da PS REM. De fato, existe grande necessidade de se conhecer os mecanismos pelos quais prejuízos no sono ou restrições de sono provocam dor.

3.1 OBJETIVO GERAL

Testar a hipótese de que o NAc medeia o efeito pró-nociceptivo induzido pela privação de sono REM através de aumento da atividade adenosinérgica sobre receptores A_{2A} e diminuição da atividade dopaminérgica sobre receptores D_2.

3.2 OBJETIVOS ESPECÍFICOS

1 - Caracterizar o efeito da privação de sono REM e subsequente sono rebote sobre a resposta nociceptiva.

2 - Testar a hipótese de que o NAc medeia o efeito pró-nociceptivo da privação de sono REM.

3 - Investigar se mecanismos dopaminérgicos, especialmente vinculados ao receptor D_2, no NAc estão envolvidos no efeito pró-nociceptivo induzido pela privação de sono REM.

4 - Investigar se os receptores A_{2A} de adenosina no NAc estão envolvidos no efeito pró-nociceptivo induzido pela privação de sono REM.

5 - Investigar se a privação de sono REM e/ou os agonistas e antagonistas adenosinérgicos e dopaminérgicos alteram a atividade motora dos animais.

6 - Estimar o efeito da privação de sono REM e dos diferentes agentes farmacológicos administrados do NAc sobre a atividade locomotora em ambiente novo (campo aberto) e em ambiente padrão (caixa de manutenção).

7- Verificar se a privação de sono REM aumenta a atividade neuronal do NAc, estimada indiretamente pela expressão da proteína c-Fos.

4. ARTIGO CIENTÍFICO

Nucleus Accumbens mediates the pronociceptive effect of REM sleep deprivation: the role of adenosine A_{2A} and dopamine D_2 receptors

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Original Article

Introduction

Persistent pain conditions and sleep disturbances can be considered public health problems worldwide (Chiu et al., 2005, Artner et al., 2013, Kim et al., 2015) and the bidirectional link between them is well established (Nicholson and Verma, 2004, Smith and Haythornthwaite, 2004). For example, sleep disturbances are present in up to 88 % of chronic pain patients (Smith and Haythornthwaite, 2004) and at least 50 % of individuals with insomnia suffer from chronic pain (Taylor et al., 2007). In fact, longitudinal studies have convincingly demonstrated that sleep deficiencies significantly increase the risk of developing chronic pain (Lyngberg et al., 2005, Mork and Nilsen, 2012). However, although there is no doubt that sleep loss increases pain in humans (Smith et al., 2007, Paul-Savoie et al., 2012, Schuh-Hofer et al., 2013) and animals (Hicks et al., 1979, Nascimento et al., 2007, Damasceno et al., 2009, Kozachik et al., 2015, Tomim et al., 2016), the mechanisms are largely unknown.

The reason why sleep disturbances are great predictors of pain development (Lyngberg et al., 2005, Mork and Nilsen, 2012) may rely on the ability of sleep loss to disrupt endogenous pain modulation, as suggested by clinical findings (Edwards et al., 2009, Tiede et al., 2010, Paul-Savoie et al., 2012). In this regard, we have recently obtained experimental data to provide a mechanistic basis for these clinical observations (Tomim et al., 2016). According to our previous data, REM (rapid eye movement) sleep deprivation (SD) increases nociceptive responses in rats by disrupting the most known endogenous pain modulation mechanism, the PAG-RVM (periaqueductal gray – rostral ventral medulla) descending system (Tomim et al., 2016). The PAG-RVM descending pathways serve as an output system that integrates afferences from multiple forebrain nuclei to modulate nociceptive transmission at the spinal dorsal horn (Millan, 2002). Some of these nuclei are engaged in both pain modulation and sleep control, however whether (and which of) these forebrain nuclei are also involved in the pronociceptive effect of SD is not known.

Nucleus accumbens (NAc), in the ventral striatum, is one of the forebrain nuclei with a major role in both pain modulation (Gear et al., 1999, Gear and Levine, 2011, Tobaldini et al., 2014) and sleep–wake cycle control (Lazarus et al., 2013). It

has been suggested that NAc promotes sleep by inhibiting, through GABAergic projections, wake-promoting nuclei in the brainstem and hypothalamus (Lazarus et al., 2013). This inhibitory output from NAc is supposed to be dependent on the balance between local dopamine and adenosine (Lazarus et al., 2013). Dopaminergic activity at inhibitory D_2 (but not D_1) receptors would inhibit NAc output, inducing wakefulness (Qu et al., 2010, Qiu et al., 2012), while adenosinergic activity at excitatory A_{2A} (but not A_1) receptors would activate this output, inducing sleep (Huang et al., 2005). Regarding pain modulation, NAc output is believed to be pronociceptive (Gear and Levine, 2011), activation of its D_2 receptors is antinociceptive (Altier and Stewart, 1999, Taylor et al., 2003) and the role of its A_{2A} receptors is not known.

Considering the above data it is possible that NAc contributes to the pronociceptive effect of REM SD, and if so, it is possible that this contribution depends on increased adenosinergic activity at A_{2A} receptors and decreased dopaminergic activity at D_2 receptors. In this study we used a pharmacological approach to functionally test these hypotheses.

Materials and Methods

Animals

The experiments were performed in male Wistar rats (250–300 g). The animals were housed five per cage, with ad libitum access to rat chow and water. They were maintained in a room with controlled 12:12-h light/dark cycle and temperature (23°C ± 2). All animal experimental procedures and protocols were approved by the local Committee on Animal Research of the Federal University of Parana and followed the guidelines of the Ethics Standards of the International Association for the Study of Pain in animals (Zimmermann, 1983).

Drugs

Qx-314 (lidocaine–ethyl bromide), a quaternary derivative of lidocaine, 2 % (Chang et al., 2014); NMDA (N-Methyl-D-aspartic acid), an excitotoxic amino acid agonist at the NMDA-type glutamate receptors, 5.5 µg (Jongen-Relo et al., 2002); Raclopride, a dopamine D_2 receptor antagonist, 5 µg (Altier and Stewart, 1998); Piribedil, a dopamine D_2 receptor agonist, 6 µg; SCH 58261, an adenosine A_{2A} receptor antagonist, 7 ng (Xia et al., 2008); CGS 21680, an adenosine A_{2A} receptor agonist, 24 ng (Font et al., 2008). Qx-314 and NMDA were administered either in NAc Core or NAc Shell. The other drugs were administered in the NAc Core. All drugs were purchased from Sigma, St Louis, MO, USA, except SCH 58261 and CGS 21680 that were obtained from Tocris Bioscience, Avonmouth, Bristol, UK. All drugs were dissolved in 0.9 % NaCl, except SCH 58261 that was dissolved in DMSO.

Stereotaxic Surgery and drug infusion

The rats were anesthetized with xylazine (3 mg/kg) and ketamine (100 mg/kg) and placed in a stereotaxic apparatus. The skull was exposed and a small hole was made to introduce a 26-gauge guide cannula into NAc Core or Shell. For NAc Core, the coordinates from bregma were: +1.3 mm from the anteroposterior (AP), lateral (L) +/−1.8 mm, and dorsoventral (DV) -5.2 mm. For NAc Shell, the coordinates from

bregma were: +1.3 mm from the AP, L +/−0.7 mm, and DV -6.1. The cannula was fixed into place with orthodontic resin (L.D. Caulk Co., Milford, DE, USA), and a small screw was fixed in the skull to ensure its immobility. After surgery, the animals received dipyrone (50 mg/kg) and enrofloxacin (5 mg/kg), and experiments were carried out 7–9 days later. After experiments, injection sites were verified by injecting Evans blue dye (1%, 0.5 µl) postmortem and performing 50-µm coronal sections (Gear and Levine, 1995, Tobaldini et al., 2014) to determine the location of the dye (Paxinos and Watson, 2007).

NMDA lesion (details bellow) was performed 20 days before experiments, in order to allow the complete recovery of behavioral outcomes (motor deficit and aggressiveness) in lesioned animals. Qx-314 was injected immediately after the first measurement of the paw withdrawal threshold after REM SD procedure, to determine its ability in reversing the pronociceptive effect of REM SD. All other drugs were injected twice: (1) immediately before the beginning of REM SD procedure, in order to test their ability to change the induction of the pronociceptive effect of REM SD and (2) immediately after the first measurement of the paw withdrawal threshold after REM SD procedure, in order to keep their effect during sleep rebound period.

NMDA Lesion

Selective bilateral lesions were performed in the NAc by injecting NMDA dissolved in NaCl 0.9% (pH 7.4) at a concentration of 5.5 µg (pH 7.2 – 7.4) (Jongen-Relo et al., 2002). For NAc core the coordinates were the same described above (injection volume 0.3µl). Three injections (0.1µl each) were performed into NAc shell throughout its dorsoventral extension: AP +1.3mm, L+/- 0.7mm, DV -6.4; -6.9; -7.9 mm 0.1µl per height. The rats assigned to the sham group were infused with NaCl 0.9%. The skin was sutured and animals observed for 3 hours, experiments were carried out 20 days later) (Jongen-Relo et al., 2002). Lesion location and extension were histologically assessed.

REM sleep deprivation (REM SD) procedure and sleep rebound period

Since most of the sleep disturbances impairs predominantly REM sleep (Brown et al., 2012), which takes place mainly in the second half of the night, we

have used the single-platform method to abolish REM sleep (Morden et al., 1967). Briefly, the rats were placed on a 6.5-cm-diameter, 10-cm-high circular platform located in a cage (23×23×35 cm) filled by water up to 1 cm of the platforms' surface. As soon as they enter in REM sleep, the rats fall off the platform, due to muscular atonia, and wake up. Therefore, SD induced by the platform method involves numerous awakenings, which predominantly affect the REM stage of the sleep. Such procedure is considered to mimic sleep fragmentation due to repeated awakenings being a useful tool to investigate the effects of sleep loss (Machado et al., 2005). The control groups were maintained under same conditions, but there was no water in the cages.

Behavioral tests (nociceptive test and open field, see below) were performed within 30 min after REM SD procedure. Animals were then returned to their home cage, where they were free for sleeping during 48 h. This period consisted in the named rebound period, during which reversion of the pronociceptive response and changes in home cage activity were assessed.

Mechanical Nociceptive Paw Withdrawal Test

Nociceptive behavioral testing was performed before, immediately after the end of the REM SD procedure and at different time points thereafter, always during the light phase (between 12:00 a.m. and 6:00 p.m.), in a quiet room maintained at 23 °C. Before the experiments, each animal was manipulated for 7 days to be habituated to the experimental manipulation. Rats did not have access to food or water during the test and each animal was used only once.

The Randall–Selitto test (Randall and Selitto, 1957) was used to assess the nociceptive mechanical paw withdrawal threshold, as a measure of the nociceptive response. In this test, a continuous pressure is applied to the dorsal surface of the rat's hind paw until the animal withdrew the paw. The nociceptive mechanical threshold was defined as the force in grams at which the rat withdrew its paw. The value was obtained from the mean of three readings made at intervals of 3 min. Data were expressed as mean $\pm$ SEM of the mechanical paw withdrawal threshold (g) at each time point.

Spontaneous locomotion in the Open Field

In order to determine the immediate effect of REM SD and different treatments on spontaneous locomotor/exploratory behavior in a novel environment, animals were tested in the open field arena 15 minutes after the end of REM SD procedure.

The open field arena consists in a circular area (1 m of diameter) limited by a 40 cm-high wall, divided into 16 squares. The animals were placed at center of the arena and allowed to explore it for 1 min. The exploratory behavior was measured by the quantification the number of crossed squares. The test was performed between 15 and 30 min after REM SD period.

Spontaneous activity in home cage

In rodents, there is a relationship between awakening and motor activity (Garcia-Rill et al., 1996) and decreased activity measured by actimetry has been used as an indirect behavioral measure of sleep in both rodents (Tang and Sanford, 2002) and humans (Townhill et al., 2016).

Motor activity measured by actimetry was performed using two different protocols with two different purposes, depending on the experiment: (1) to monitor the effect of REM SD (and different pharmacological treatments at NAc) in the sleep time under standard environmental conditions (community), animals were housed four per cage and home cage motor activity was monitored before REM SD (for 24h) and during sleep rebound period (for 48h); (2) to ensure that the doses of the agonists and antagonists used in this study were effective to change sleep time as indicated by literature (Satoh et al., 1999, Barik and de Beaurepaire, 2005, Qu et al., 2010, Huang et al., 2011, Qiu et al., 2012), animals were individually housed and their activity was measured for 24h after drug or vehicle administration.

Passive infrared motion captors were placed over the cages and connected to a computerized data acquisition system (National Instruments, Austin, TX, USA). Activity records were analyzed with the LabVIEW software package (National Instruments, Austin, TX, USA). Total activity counts were recorded in a 12 h light/12 h dark cycle.

Histological sample preparation

The rats were anesthetized by an intra-muscular injection of xylazine (3mg/kg) and ketamine (100 mg/kg) and transcardially perfused with saline followed by 4 % paraformaldehyde in 0.1-Mphosphate buffer, pH 7.4. Brains were removed and immersed in paraformaldehyde at 4 °C for a week, in 30 % sucrose solution for another week and stored at −80°C until sectioning. Four sections (30 µm), between bregma +1.44 and +1.20 mm, were sliced per animal.

Cresyl Violet staining

Cresyl Violet, a cationic dye that stains Nissl corpuscles present in the cell body and dendrites of neurons (Ovalle, 2013) was used in order to determine the extension of NMDA lesions. The sections were mounted on to gelatin-coated slices, passed through a series of ethanol solutions of descending concentration (3 min in each of 100%, 95%, and 70% ethanol in water) and stained for ~5 min with cresyl violet (0.05% aqueous cresyl violet, 2mM acetic acid, and 5mM formic acid in water). After staining, sections were rinsed in water and 70% ethanol; differentiated in 95% ethanol with acetic acid; dehydrated in ascending concentrations of ethanol–xylene and cover slipped.

c-Fos Immunohistochemistry

c-Fos protein is rapidly and transiently expressed in stimulated neurons in response to elevation of intracellular calcium (Lerea et al., 1992, Coderre et al., 1993). Therefore, we have quantified c-Fos expression in an attempt to indirectly estimate the effect of REM SD on neuronal activation at NAc. Free-floating sections were rinsed in 0.1M phosphate-buffered saline (PBS) and treated with 0.5% H2O2 in 0.1M PBS for 30 min to suppress endogenous peroxidase activity. Tissue sections were incubated overnight at 4^0C with rabbit anti-c-Fos primary antibody (#AB038; Chemicon, Temecula, CA; 1:500 in phosphate-buffered saline plus 0.3%Triton X-100) and then incubated with a biotin-conjugated secondary antibody (#PK4001; Vector Laboratories, Burlingame, CA; 1:200) for 2 hours at room temperature. After several washes with phosphate-buffered saline, the antibody complex was localized

using the ABC system (#PK4001; Vectastain ABC Elite kit, Vector Laboratories, Burlingame, CA) followed by reaction with 3,30-diaminobenzidine with nickel enhancement. The sections were then mounted onto gelatin coated slices and cover slipped after dehydration in ascending concentrations of ethanol-xylene solutions.

Quantification of excitotoxic lesion and c-Fos immunoreactive cells

The slices were digitized with a microscope scanner (Axio Imager Z2, Carl Zeiss, Jena, DE) coupled to an imaging system (Metasystems, Altlussheim, DE). Quantification of NAc lesion and c-Fos immunoreactive (c-Fos-ir) cells was performed automatically by optical density using ImageJ 1.37c (Public Domain) image analysis software.

Statistical Analysis

Data from nociceptive tests were analyzed by repeated-measures (time) analysis of variance (ANOVA) with sleep condition (REM SD or control procedure) as factor for naïve animals and sleep condition and treatment (drug or vehicle) as factors for all other groups. Data from actimetry were analyzed in group housed rats by repeated-measures (time) ANOVA with sleep condition as factor and in individually housed rats by two way ANOVA with treatment and phase (light or dark) as factors. Data from Cresyl Violet staining were analyzed by two-way ANOVA with sleep condition and treatment (NMDA lesion or sham) as factors. Data from c-Fos immunohistochemistry were analyzed by one-way ANOVA. Data from open field were analyzed by t test or by two-way ANOVA with sleep condition and treatment as factors. All post hoc contrasts, when appropriate, were performed using Tukey's test. The level for statistical significance was $p<0.05$. STATISTICA® software (StatSoft, Tulsa, OK, USA) was used to perform data analysis. SigmaPlot® software (Systat Software, San Jose, CA, USA) was used to perform graphical representation. Data are plotted in figures as mean ± SEM.

Results

The pronociceptive effect of REM sleep deprivation and its progressive reversion during sleep rebound

The nociceptive response was measured before (baseline) and immediately, 15 and 30 min after REM SD or control procedure. Animals were then returned to their home cage, where they were free for sleeping during 48 h. This period consisted in the named rebound period, during which reversion of the pronociceptive response and changes in home cage activity was assessed.

REM SD for 24 h significantly increased nociceptive response, as demonstrated by the decrease in mechanical nociceptive paw withdrawal threshold. Sleep rebound led to a progressive reversion of the pronociceptive effect of REM SD, but a complete recover was not seen, even after 48h of sleep rebound (Figure 1 A, repeated-measures ANOVA – sleep condition (REM SD or control procedure): $F(1,10) = 1180.90$, $p < 0.0001$); sleep condition x time: $F(7,70) = 181.89$, $p < 0.0001$- followed by Tukey's post hoc, $p < 0.05$).

After 24h of REM SD home cage activity was decreased in the dark, but not in the light phase. Sleep rebound for 24h appears not to be enough to recover dark phase activity, since it remains decreased in the next dark phase, which ended the 48 h period of sleep rebound (Figure 1 B, repeated-measures ANOVA, sleep condition: $F(1, 2) = 6.00$, $p = 0.13$); sleep condition x time: $F(5, 10) = 1.55$, $p = 0.25$ - followed by Tukey's post hoc, $p < 0.05$).

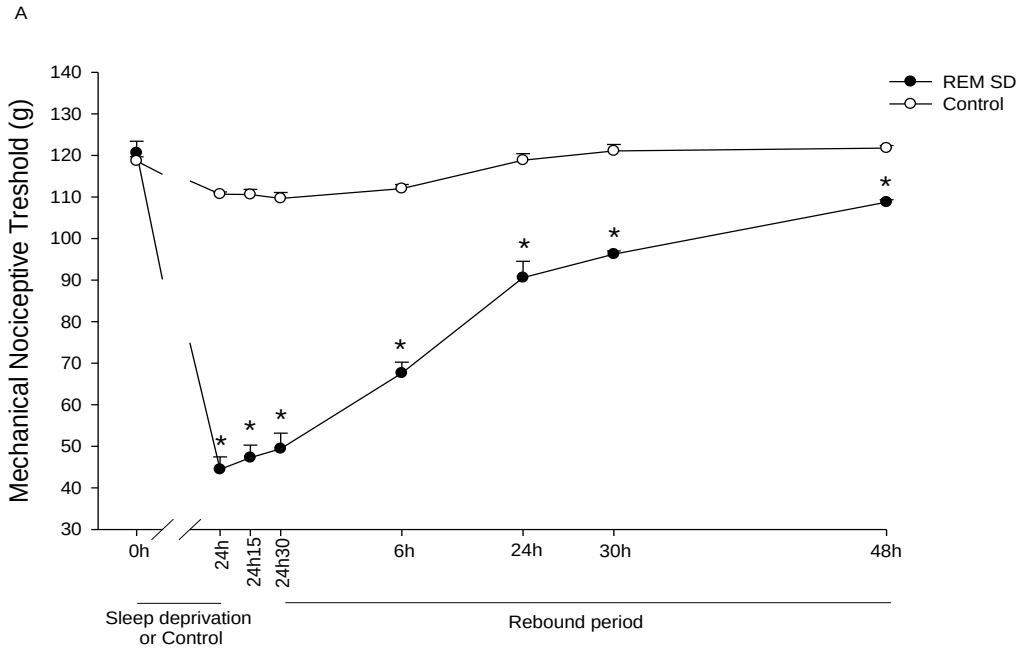
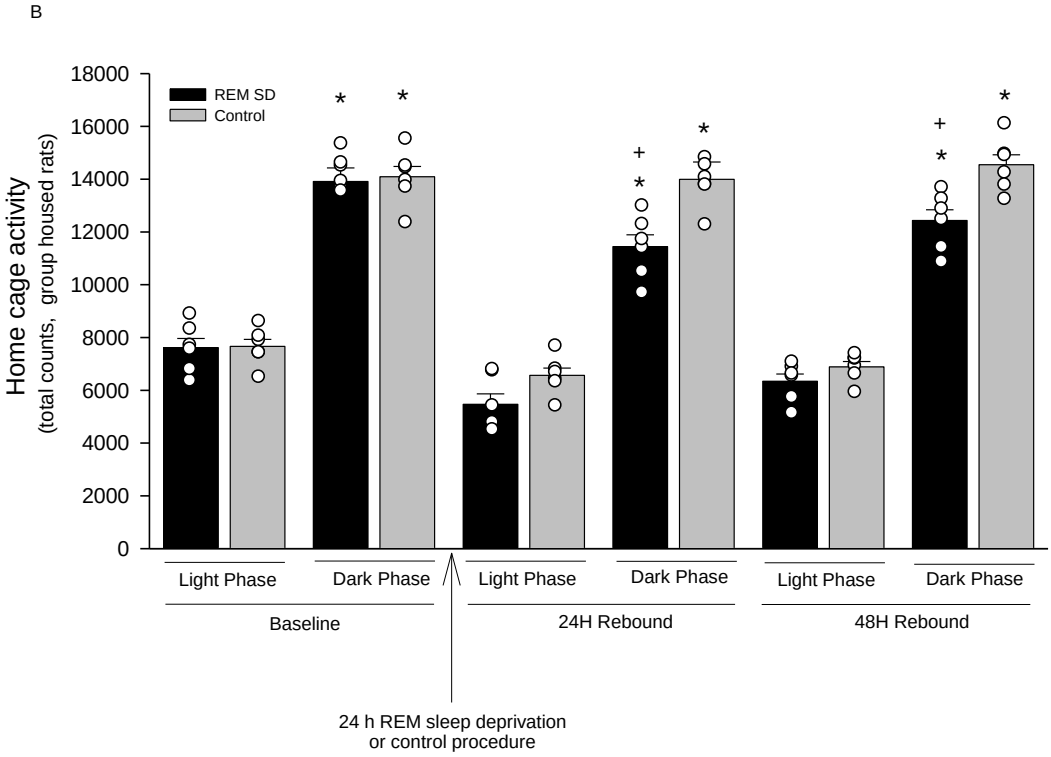


Figure 01: **Effect of REM sleep deprivation and sleep rebound on the nociceptive response and motor activity**. A- The mechanical nociceptive threshold was significantly decreased after 24 h of REM sleep deprivation and did not return to the basal level even after 48 h of sleep rebound (repeated measures ANOVA, with one within-subjects factor (time) and one between-subjects factors (sleep condition). The symbol **"*"** indicates a mechanical nociceptive threshold significantly lower than that of the control group within the same time point (Tukey's post hoc test, $p < 0.05$). In this and in subsequent figures data are expressed as mean ± S.E.M. of the mechanical paw withdrawal threshold obtained from 6-8 animals per group. See methods for additional details regarding experimental protocol data and analysis.



B - After 24 h of REM sleep deprivation, home cage activity in group housed rats (four rats per cage) significantly decreased during two consecutive dark phases (repeated measures ANOVA, with one within-subjects factor (time) and one between-subjects factors (sleep condition). The symbol "*" indicates a significantly greater activity during the dark phase than during light phase, the symbol "+" indicates dark phase activity significantly lower than that of the control group (Tukey's post hoc test,p

< 0.05), the circles indicate individual values. Data are expressed as mean ± S.E.M. of total activity counts obtained from 6 cages per group (n = 6). See methods for additional details regarding experimental protocol data and analysis.

The role of NAc in the pronociceptive effect of REM sleep deprivation

The excitotoxic lesion induced by previous administration of NMDA either into the NAc core (Figure 2 A, repeated-measures ANOVA – sleep condition: $F(1, 14) = 11319.00$, $p < 0.0001$; treatment (NMDA or sham lesion): $F(1, 14) = 15386.00$, $p < 0.0001$; sleep condition x treatment: $F(1, 14) = 11192.00$, $p < 0.0001$; sleep condition x treatment x time: $F(5, 70) = 1034.00$, $p < 0.0001$) or into NAc shell (Figure 2 C, repeated-measures ANOVA – sleep condition: $F(1, 16) = 3630.30$, $p = < 0.0001$; treatment: $F(1, 16) = 5164.30$, $p < 0.0001$; sleep condition x treatment: $F(1, 16 = 3589.30$, $p < 0.0001$; sleep condition x treatment x time: $F(5, 80) = 1176.40$, $p < 0.0001$) prevented the pronociceptive effect of REM SD (Tukey's test, $p > 0.05$, in all time points analyzed), suggesting that NAc is necessary for this effect.

Qualitative assessment of the home cage activity (two cages per group, with four animals each) suggests that NMDA lesion either in NAc core (Figure 2 B) or in NAc shell (Figure 2 D) increases dark phase activity in REM sleep deprived animals (indicated by ↑).

NMDA administration significantly decreased the number of neurons both in NAc core (Two way ANOVA – sleep condition: $F(1, 10) = 3.01$, $p = 0.112$; treatment: $F(1, 10) = 13.35$, $p = 0.004$; sleep condition x treatment: $F(1, 10) = 4.46$, $p = 0.608$) and shell (Two way ANOVA – sleep condition: $F(1, 8) = 2,97$, $p = 0.123$; treatment: $F(1, 8) = 8.58$, $p = 0.019$; sleep condition x treatment: $F(1, 8) = 4.326$, $p = 0.071$), representatives sections of NAc core and shell are depicted in Figure 2 E. Lesions are evident and restricted to NAc core or shell. The number of neurons was largely decreased in lesion groups and the remaining neurons were smaller than those of sham groups.

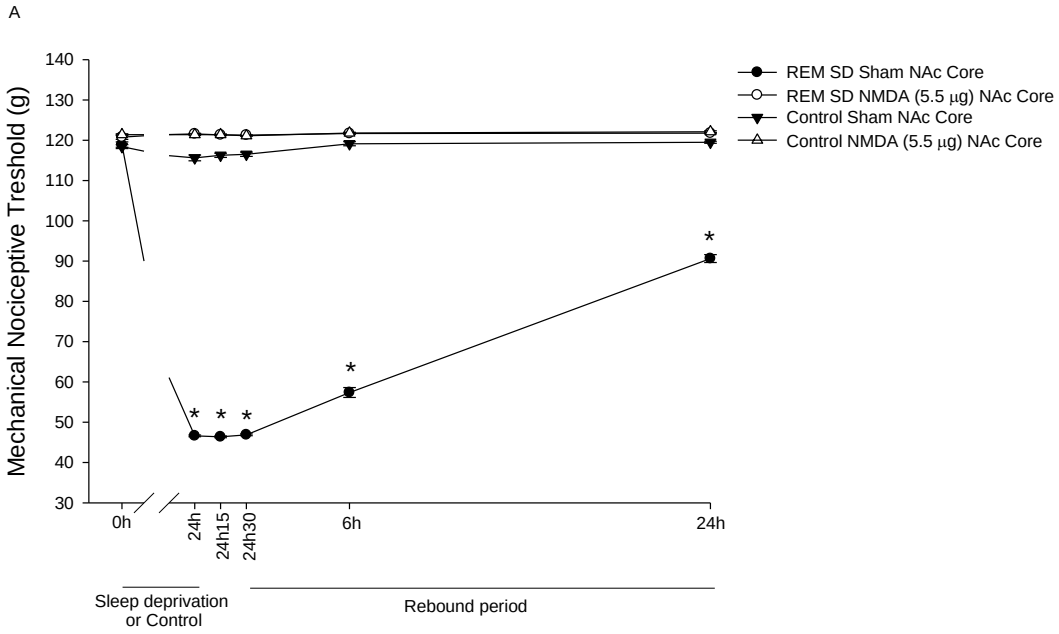
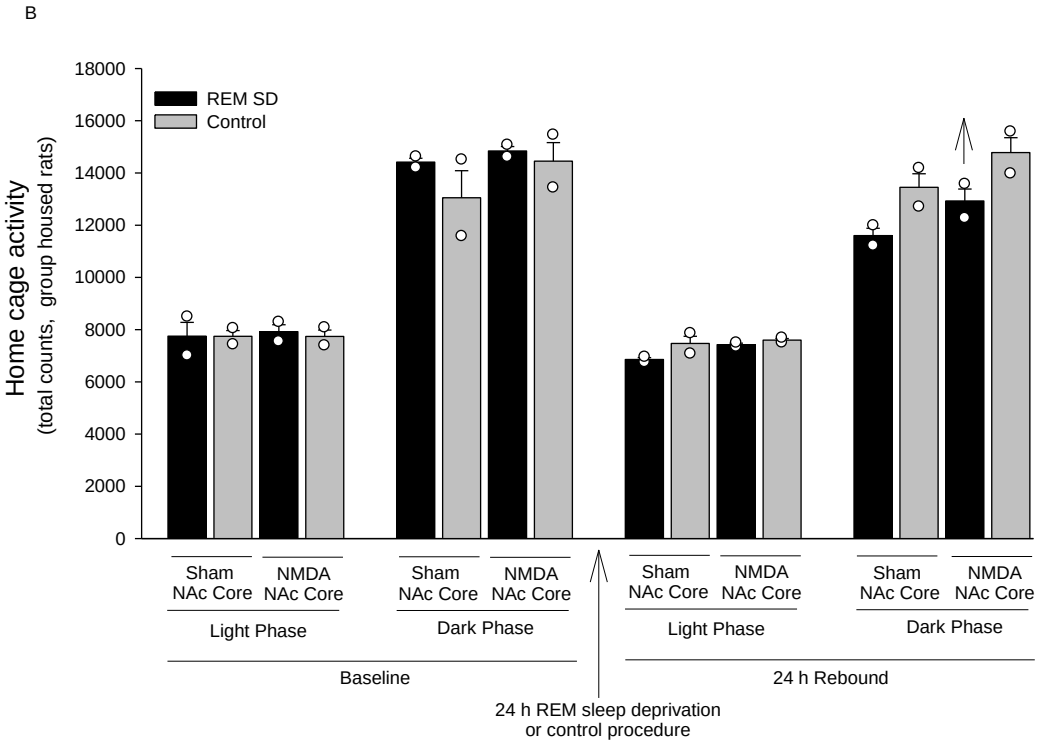
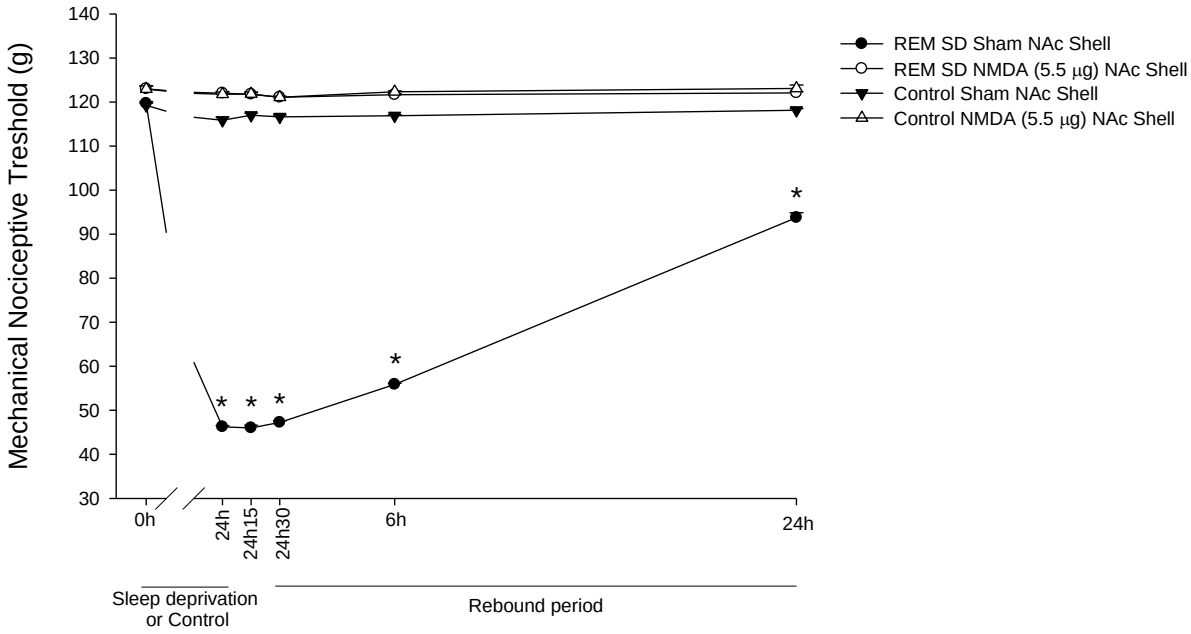


Figure 02: Effect of NAc lesion on the pronociceptive effect of REM sleep deprivation. A- Previous (20 days) administration of NMDA (5.5 µg/0.3µl) into NAc core prevented the decrease in mechanical nociceptive threshold induced by REM sleep deprivation (repeated measures ANOVA, with one within-subjects factor (time) and two between-subjects factors (sleep condition and treatment). The symbol "*" indicates a mechanical nociceptive threshold significantly lower than that of the other groups within the same time point (Tukey's post hoc test, $p < 0.05$).



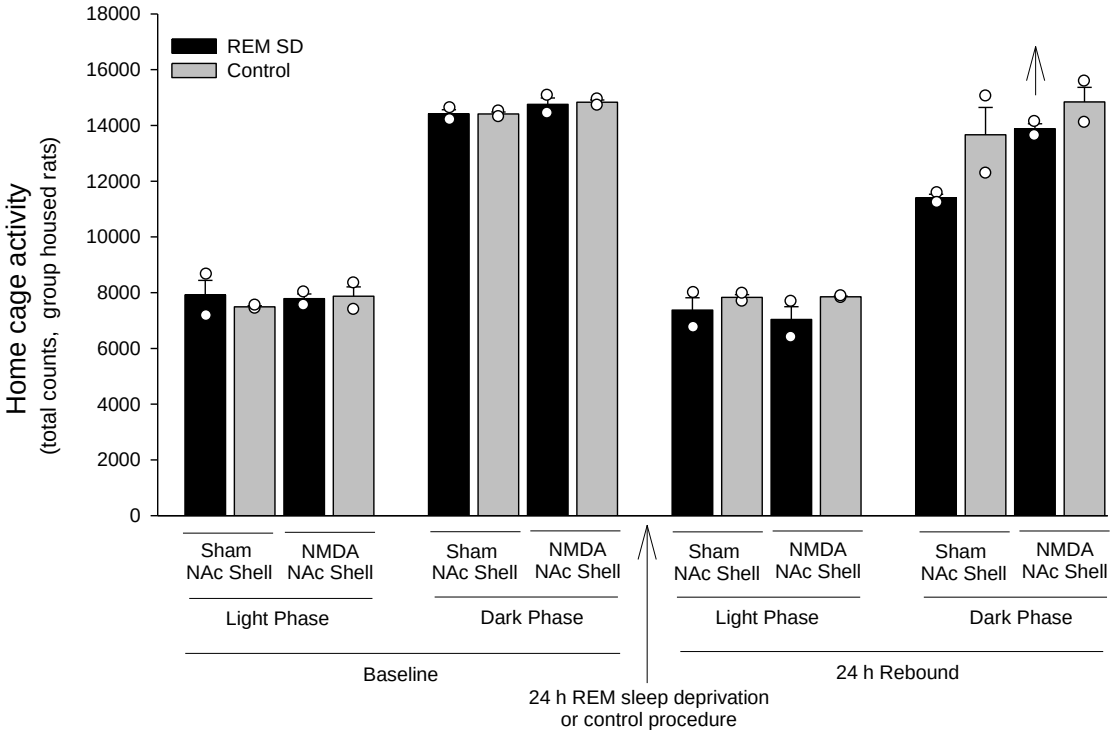
B- Qualitative assessment of home cage activity in group housed rats (four rats per cage, two cages per group) was compatible with increased home cage activity during the dark phase in sleep deprived rats submitted to NMDA lesion at NAc core (the circles indicate individual values and the arrow the suggested alteration). See methods for additional details regarding experimental protocol.

C

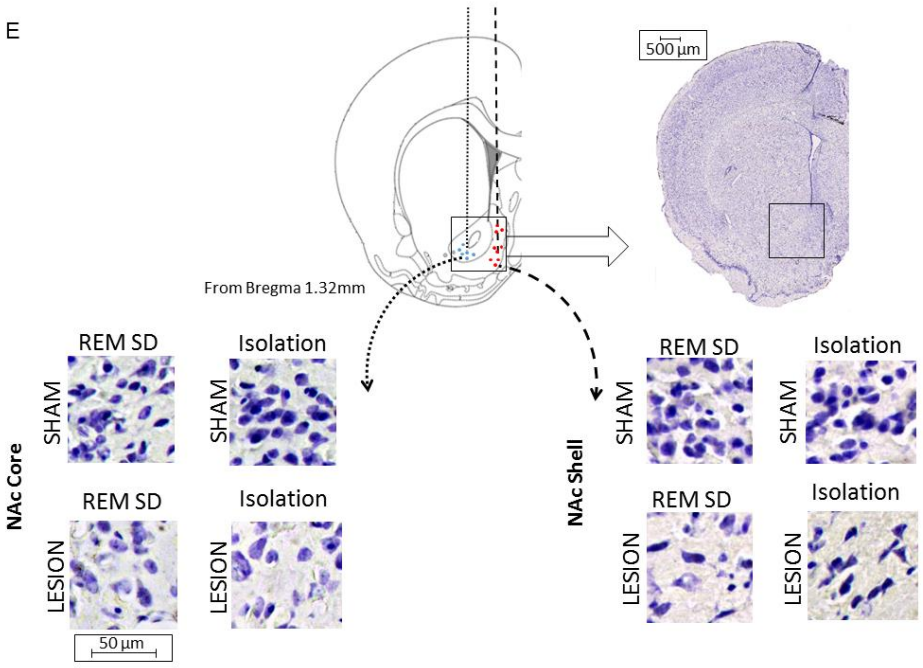


C- Previous (20 days) administration of NMDA (5.5 µg/0.3µl, three injections of 0.1 µl throughout its dorsoventral extension) into NAc shell prevented the decrease in mechanical nociceptive threshold induced by REM sleep deprivation (repeated measures ANOVA, with one within-subjects factor (time) and two between-subjects factors (sleep condition and treatment). The symbol "*" indicates a mechanical nociceptive threshold significantly lower than that of the other groups within the same time point (Tukey's post hoc test, $p < 0.05$).

D



D- Qualitative assessment of home cage activity in group housed rats (four rats per cage, two cages per group) was compatible with increased home cage activity during the dark phase in sleep deprived rats submitted to NMDA lesion at NAc shell (the circles indicate individual values and the small arrows the suggested alteration).



E – Representative photomicrographs of NMDA lesions of the NAc core (left) and NAc shell (right). Photomicrographs show cresyl violet-stained coronal sections of rat brains with selective NAc core or shell lesions. Diagrammatic representation of cross-sections from the atlas of Paxinos and Watson (2007): blue circles represent injection sites in NAc core; red circles represent injection sites in NAc shell. The numbers below the brain diagrams represent the atlas frontal coordinates in millimeters posterior to bregma.

The acute blockade of NAc by the administration of Qx-314 after REM SD either into the NAc core (Figure 3 A, repeated-measures ANOVA – sleep condition: $F(1, 20) = 1955.90$, $p < 0.0001$; treatment (Qx-314 or vehicle): $F(1, 20) = 396.88$, $p < 0.0001$; sleep condition x treatment: $F(1, 20) = 506.21$, $p < 0.0001$; sleep condition x treatment x time: $F(7, 140) = 131.88$, $p < 0.0001$) or NAc shell (Figure 3 C repeated-measures ANOVA – sleep condition: $F(1, 23) = 291.51$, $p < 0.0001$; treatment (Qx-314 or vehicle): $F(1, 23) = 33.70$, $p < 0.0001$; sleep condition x treatment: $F(1, 23) = 28.56$, $p < 0.0002$; sleep condition x treatment x time: $F(7, 161) = 23.82$, $p < 0.0001$) reversed the pronociceptive effect (Tukey's test, $p < 0.05$), suggesting that continuous NAc activity contributes to the maintenance of the pronociceptive effect of REM SD.

Qualitative assessment of home cage activity suggests that acute blockade of NAc core (Figure 3 B), but not NAc shell (Figure 3 D), increases dark phase activity in both REM sleep deprived and control animals (indicated by ↑). Based on the better results obtained with acute blockade of NAc core, this region was chosen for the following experiments.

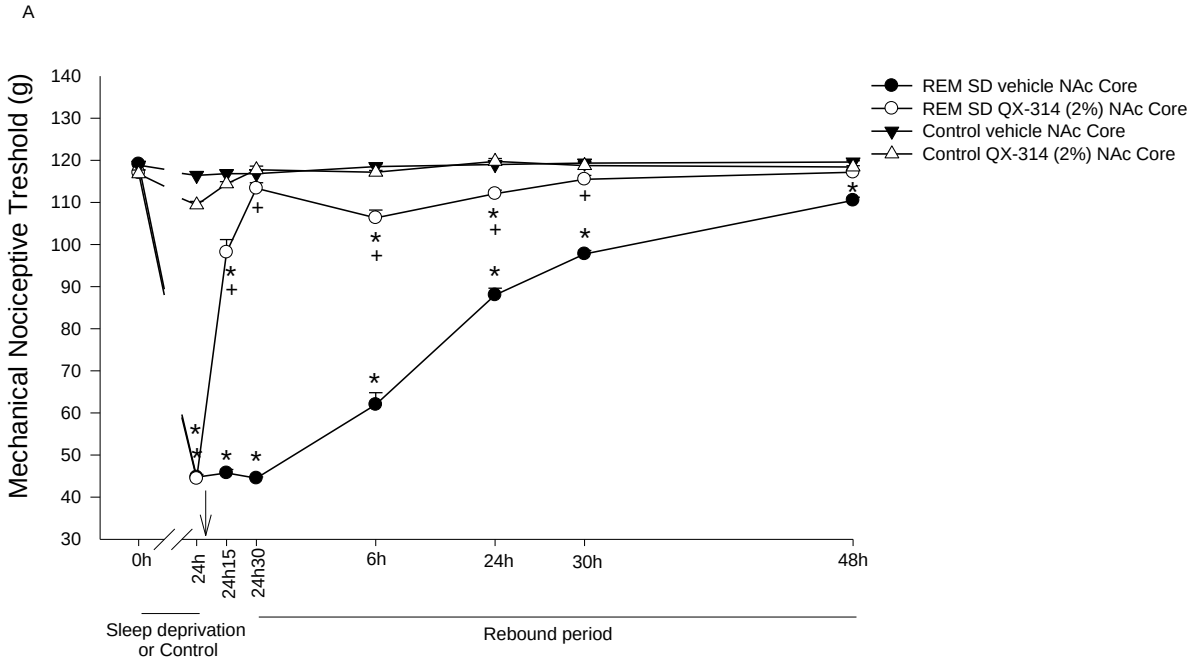
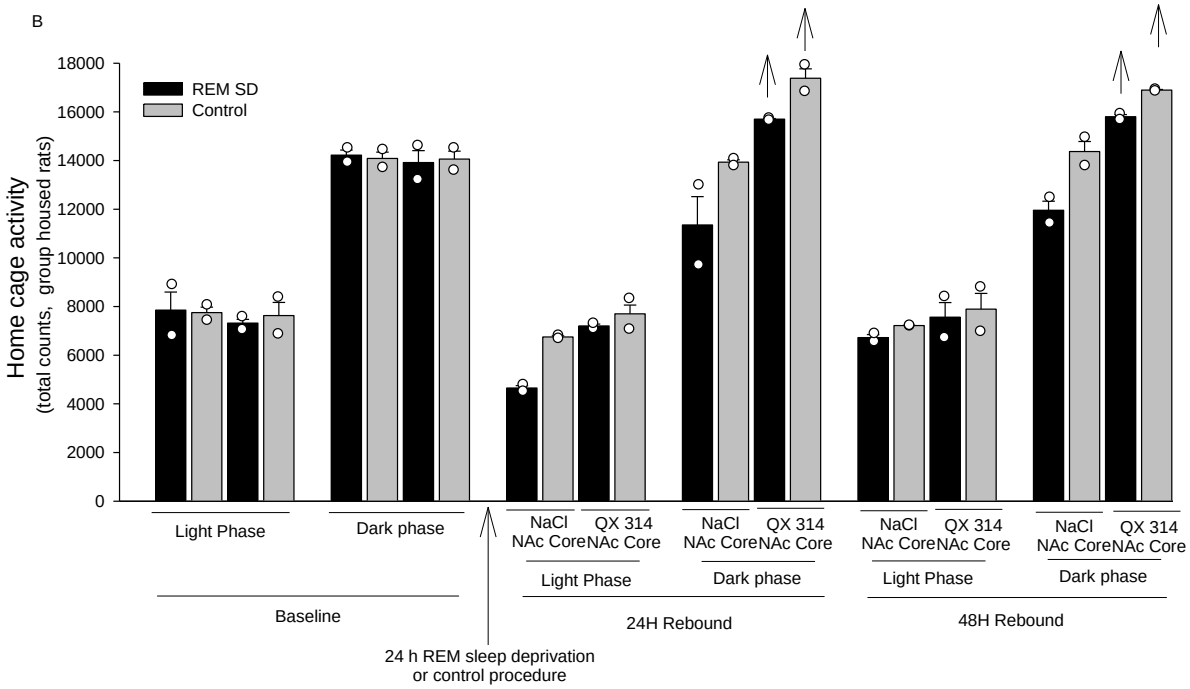
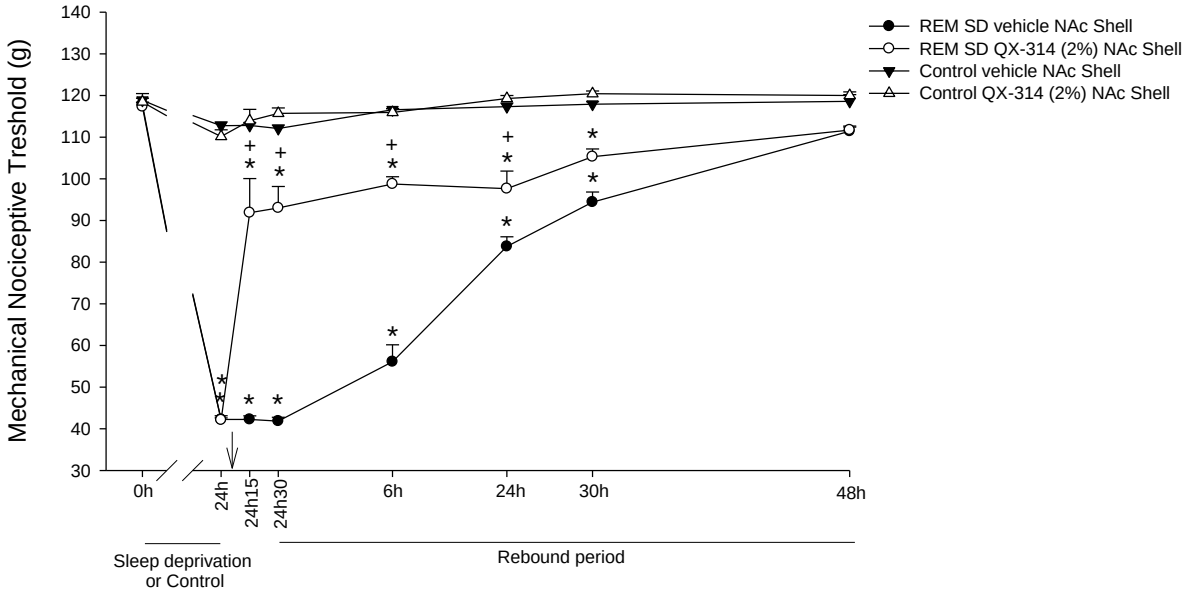


Figure 03: Effect of acute blockade of NAc activity on the pronociceptive effect of REM sleep deprivation. A- Administration of Qx-314 (2%) into NAc core reversed the decrease in mechanical nociceptive threshold induced by REM sleep deprivation (repeated measures ANOVA, with one within-subjects factor (time) and two between-subjects factors (sleep condition and treatment). The symbol "*" indicates a mechanical nociceptive threshold significantly lower than that of control groups within the same time point and "+" indicates a mechanical nociceptive threshold significantly greater than that of the sleep deprived rats receiving vehicle within the same time point (Tukey's post hoc test, $p < 0.05$). Arrows indicate when Qx-314 was injected.



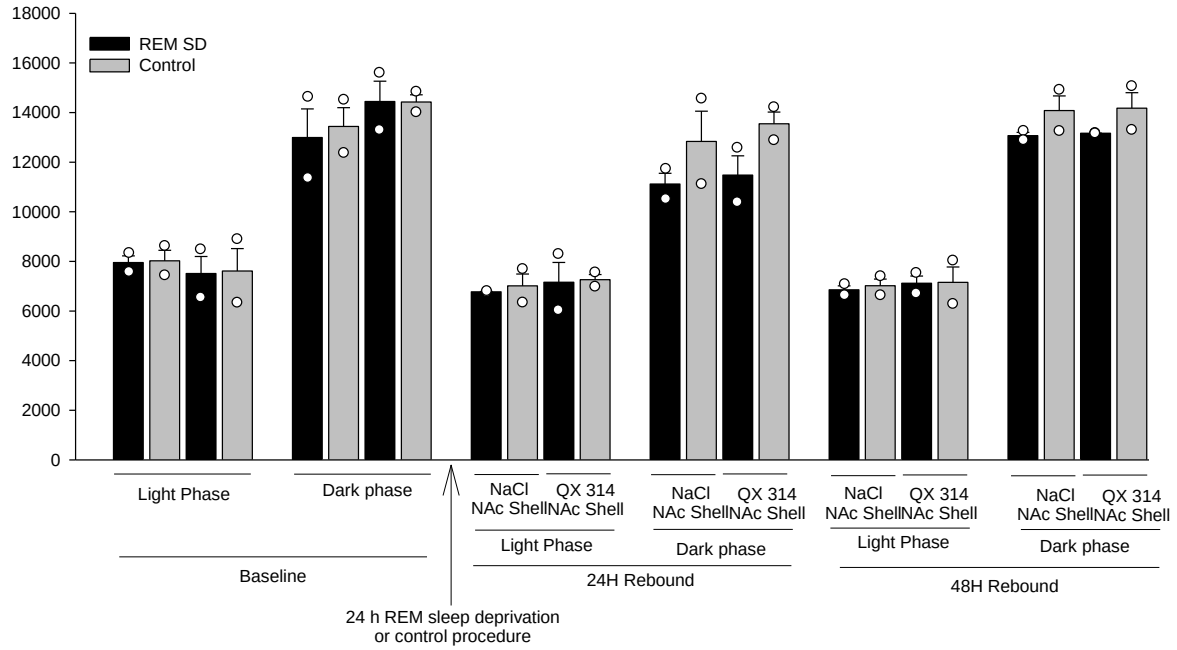
B- Qualitative assessment of home cage activity in group housed rats (four rats per cage, two cages per group) was compatible with increased home cage activity during the dark phase in sleep deprived and control rats treated with Qx-314 at NAc core (the circles indicate individual values and the small arrows the suggested alteration).

C



C- Administration of Qx-314 (2%) into NAc shell partially reversed the decrease in mechanical nociceptive threshold induced by REM sleep deprivation (repeated measures ANOVA, with one within-subjects factor (time) and two between-subjects factors (sleep condition and treatment). The symbol "*" indicates a mechanical nociceptive threshold significantly lower than that of the control groups within the same time point and "+" indicates a mechanical nociceptive threshold significantly greater than that of the sleep deprived rats receiving vehicle within the same time point (Tukey's post hoc test, $p < 0.05$). Arrows indicate when Qx-314 was injected.

D



D- Qualitative assessment of home cage activity in group housed rats (four rats per cage, two cages per group) suggests that Qx-314 at NAc shell did not change home activity during the dark phase in sleep deprived and control rats (the circles indicate individual values and the small arrows the suggested alteration).

The role of NAc adenosine A_{2A} and dopamine D_2 receptors in the animals activity over a 12 h light/12 h dark cycle.

Home cage activity in individually housed rats was measured by actimetry over 24 h (12 h light/12 h dark cycle) to assess the effect of adenosine A_{2A} and dopamine D_2 receptor agonists and antagonists at the doses used in the present study in animals' activity.

The administration into the NAc Core of the adenosine A_{2A} receptor agonist, CGS 21680, decreased, while that of the antagonist, SCH 58261, increased activity measured by actimetry (Figure 4 A two-way ANOVA – treatment (drug or vehicle): $F(2, 30) = 18.35$, $p < 0.00001$; treatment x time: $F(2, 30) = 5.97$, $p = 0.006$). Specifically, the A_{2A} receptor agonist decreased activity during the dark phase, abolishing the basal difference between light and dark phase activity (Tukey's test, $p = 0.339$). The A_{2A} receptor antagonist significantly increased activity during the dark phase compared with vehicle and agonist treated animals (Tukey's test, $p < 0.001$).

The administration into the NAc Core of the dopamine D_2 receptor agonist, piribedil, increased, while that of the antagonist, raclopride, decreased activity measured by actimetry (Figure 4 B two-way ANOVA – treatment: $F(2, 24) = 7.06$, $p = 0.003$; treatment x time: $F(2, 24) = 1.42$, $p = 0.259$). Specifically, the D_2 receptor agonist increased activity during the light phase, abolishing the basal difference between light and dark phases (Tukey's test, $p = 0.25$). The D_2 receptor antagonist significantly decreased activity during the dark phase compared with vehicle and agonist treated animals (Tukey's test, $p < 0.05$) and abolished the basal difference between light and dark phases (Tukey's test, $p = 0.90$).

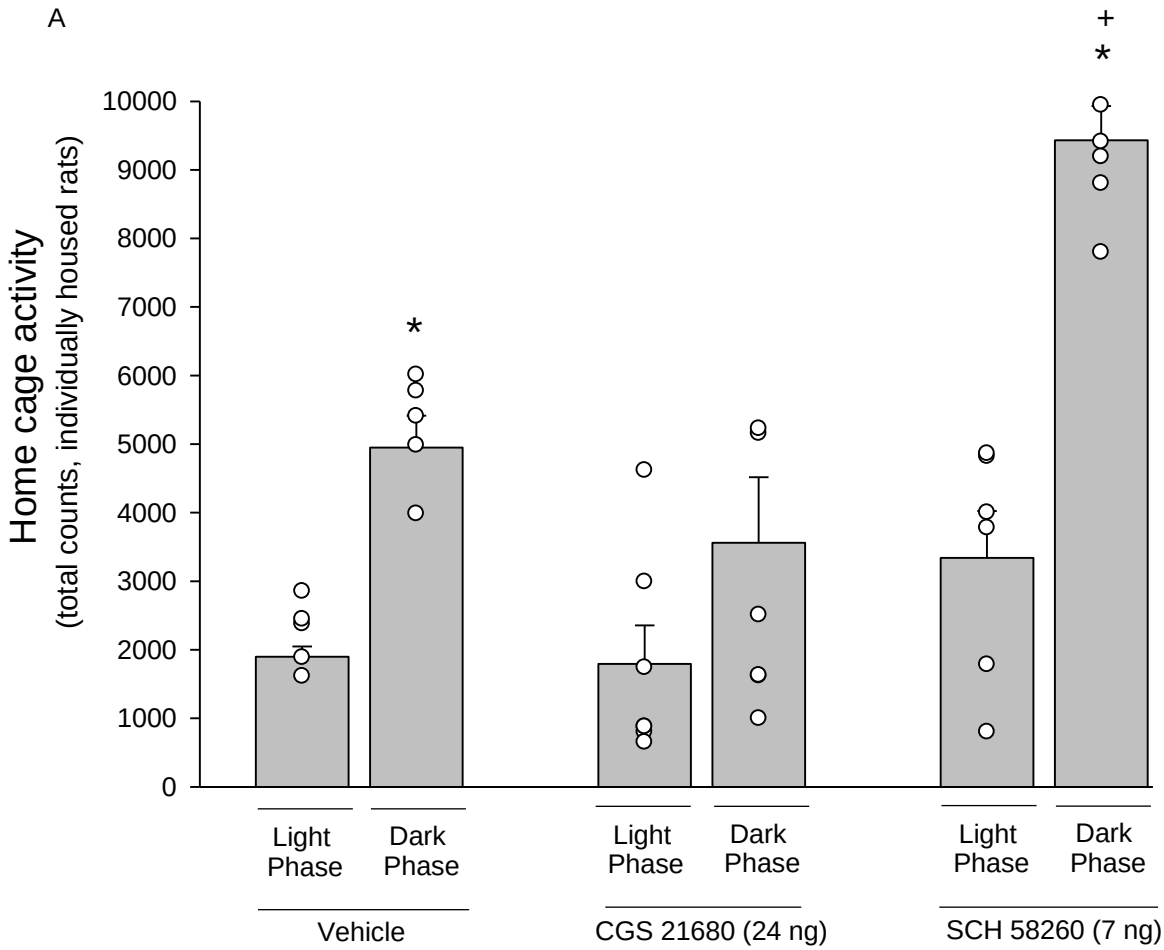
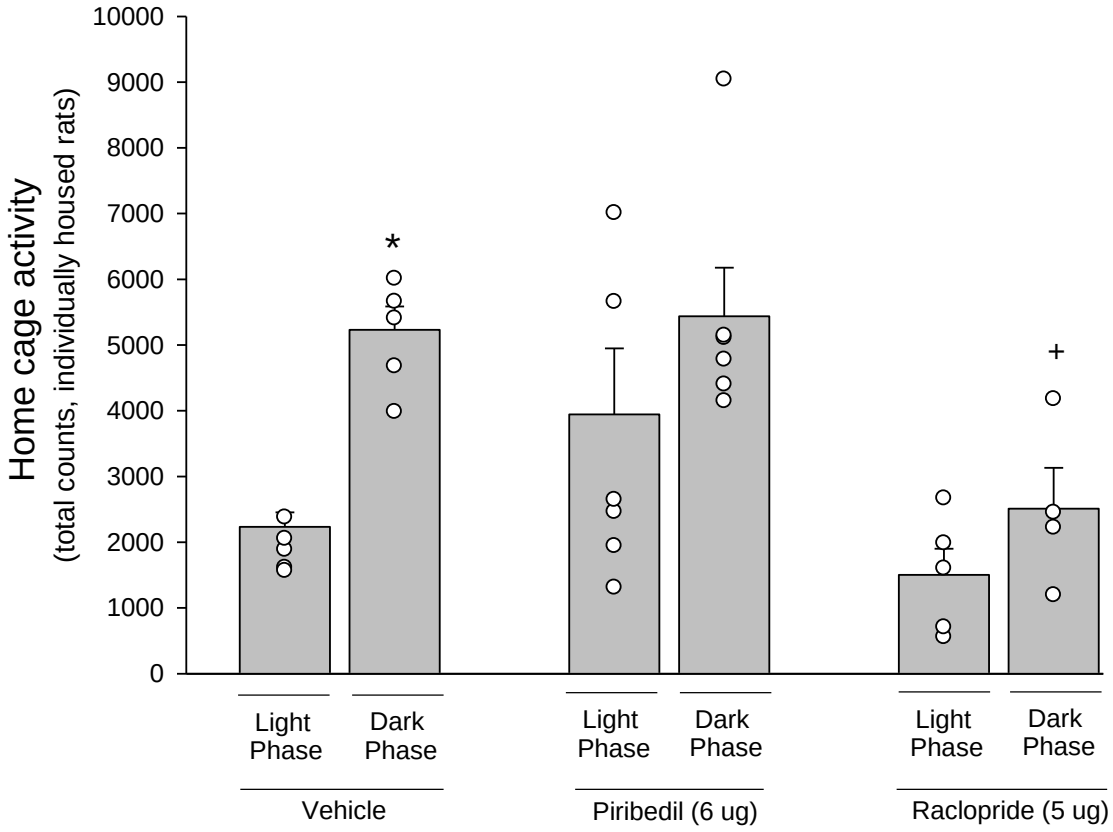


Figure 04: Effect of NAc core A_{2A} adenosinergic and D_2 dopaminergic activity on home cage activity of individually housed rats. A- The administration into the NAc core of the A_{2A} receptor agonist (CGS 21680) decreased while that of the antagonist (SCH 58260) increased home cage activity in individually housed rats (repeated measures ANOVA, with one within-subjects factor (time) and one between-subjects factors (treatment). The symbol "*" indicates activity significantly greater during the dark phase than that during light phase within treatment group, the symbol "+" indicates dark phase activity significantly greater than that of the other groups (Tukey's post hoc test, $p < 0.05$), circles indicate individual values. Microinjections were performed immediately before experiment (activity measurement).

B



B- The administration into the NAc core of the D_2 receptor agonist (Piribedil) increased light phase activity while that of the antagonist (raclopride) decreased activity either in the light or dark phases (repeated measures ANOVA, with one within-subjects factor (time) and one between-subjects factors (treatment). The symbol "*" indicates activity significantly greater during the dark phase than that during light phase within treatment group, the symbol "+" indicates dark phase activity significantly lower than that of the other groups (Tukey's post hoc test, $p < 0.05$), circles indicate individual values. Microinjections were performed immediately before experiment (activity measurement). See methods for additional details regarding experimental protocol data and analysis.

The role of NAc adenosine A_{2A} receptors in the pronociceptive effect of REM sleep deprivation and in its progressive reversion during sleep rebound

The administration of the adenosine A_{2A} receptor agonist CGS 21680 into the NAc Core did not affect the immediate pronociceptive effect of REM SD (Figure 5 A, repeated-measures ANOVA – sleep condition: $F(1, 21) = 1081.20$, $p < 0.0001$; treatment (CGS 21680 or vehicle): $F(1, 21) = 0.023$, $p = 0.880$; sleep condition x treatment: $F(1, 21) = 6.97$, $p = 0.015$; sleep condition x treatment x time: $F(7, 147) = 11.83$, $p < 0.0001$). However, while in vehicle treated animals the nociceptive threshold increased progressively during the rebound period, in CGS 21680 treated ones it did not further increase after 24 hour of sleep rebound (Tukey's test, $p <$

0.0001). This last finding suggests that increased activity at NAc adenosine A_{2A} receptors impairs the reversion of the pronociceptive effect during sleep rebound.

Qualitative assessment of the home cage activity (Figure 5 B) suggests that the adenosine A_{2A} receptor agonist decreased activity in the light and dark phases in REM sleep deprived animals and in the first dark phase after its administration in control animals (indicated by↓).

The administration of the adenosine A_{2A} receptor antagonist SCH 58261 into the NAc Core prevented the pronociceptive effect of REM SD (Figure 5 C, repeated-measures ANOVA – sleep condition: $F(1, 17) = 157.48$, $p < 0.0001$; treatment (drug or vehicle): $F(1, 17) = 78.82$, $p < 0.0001$; sleep condition x treatment: $F(1, 17) = 125.62$, $p < 0.0001$; sleep condition x treatment x time: $F(7, 119) = 12.54$, $p < 0.0001$. Post hoc analysis using Tukey's test indicated that REM SD failed to induce a pronociceptive effect in SCH 58261 treated animals $p > 0.05$), suggesting that NAc adenosine A_{2A} receptors are necessary to the pronociceptive effect of REM SD.

Qualitative assessment of the home cage activity (Figure 5 D) suggests that the adenosine A_2A receptor antagonist increased activity in the dark phases in both REM sleep deprived and control animals (indicated by↑).

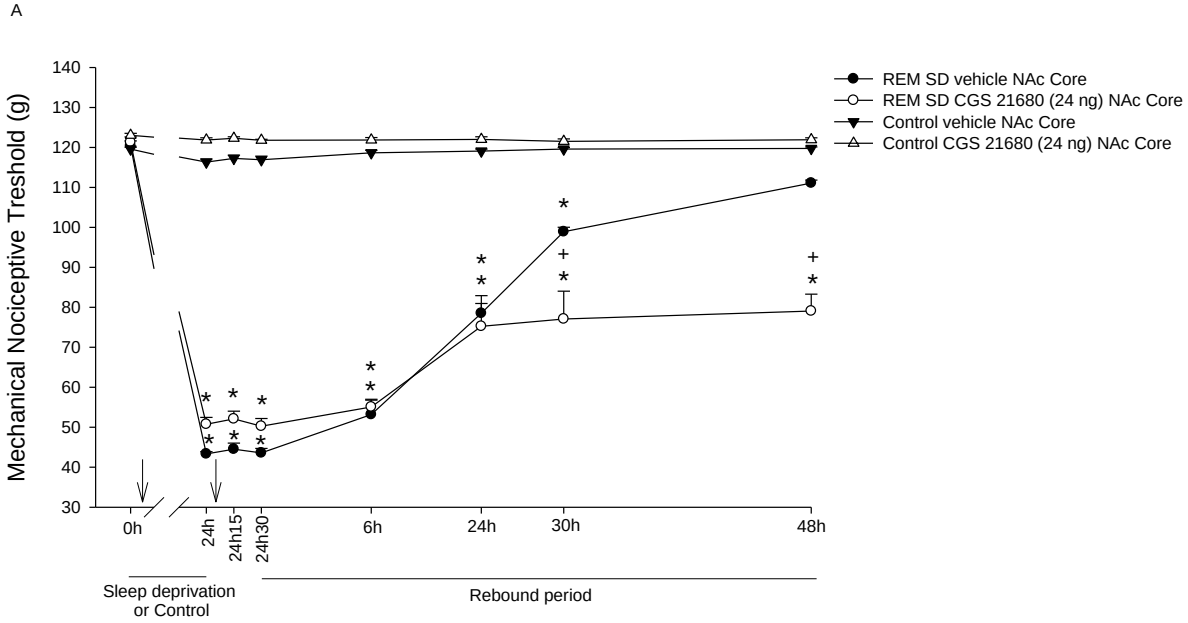
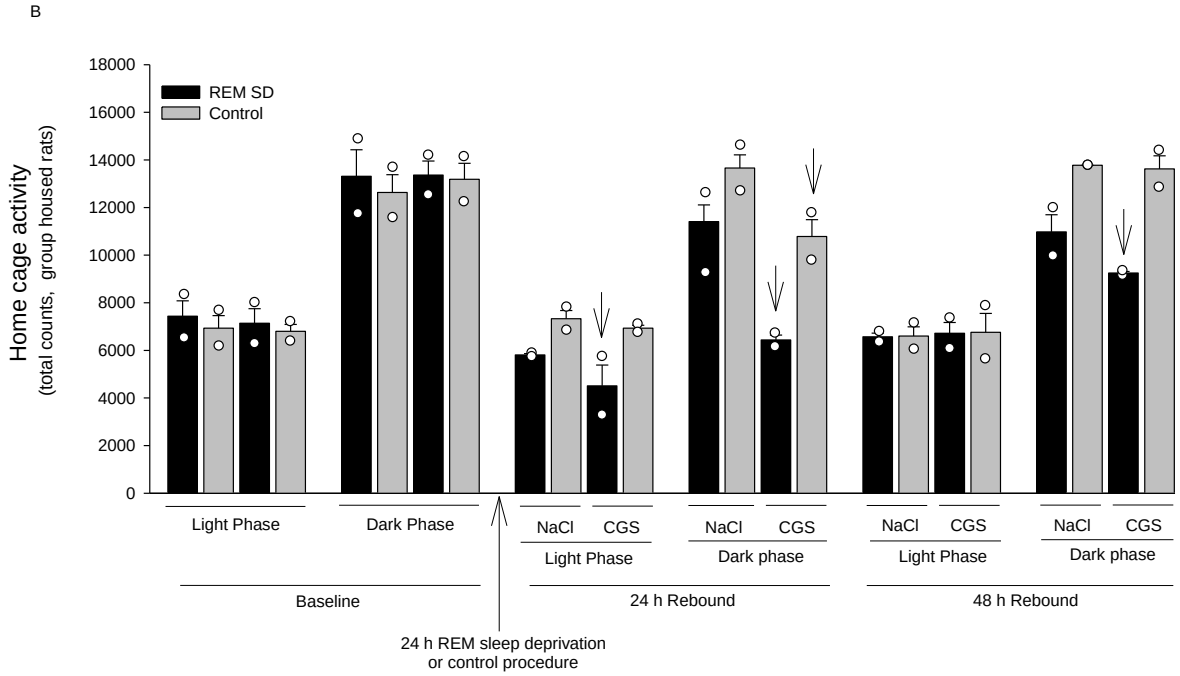
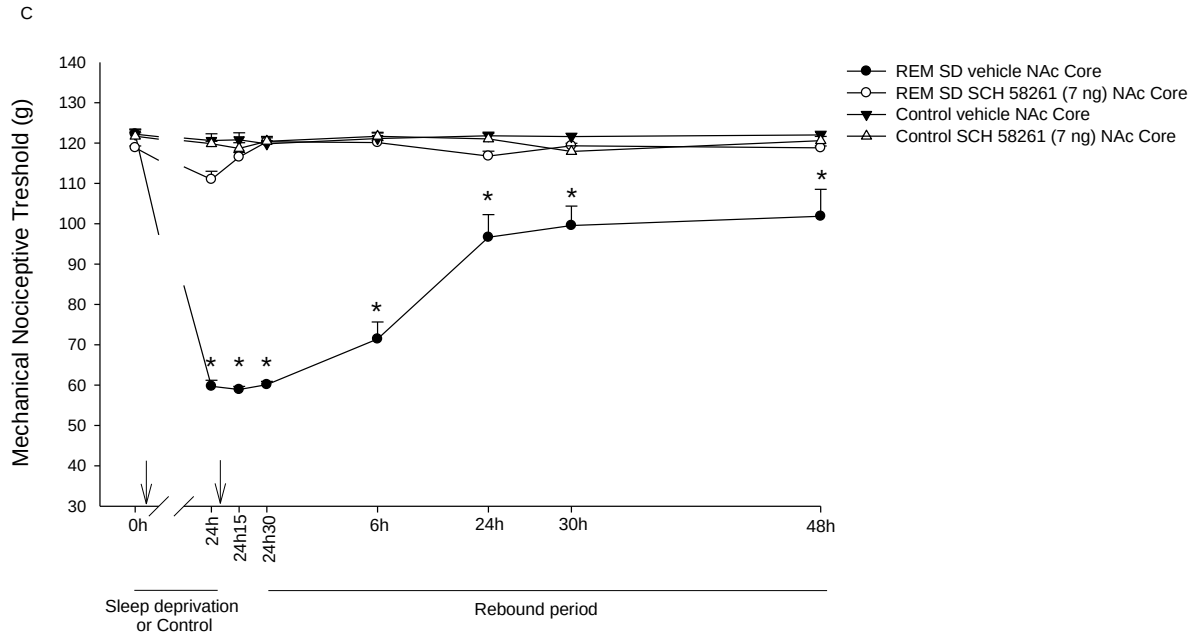


Figure 05: Effect of agonism and antagonism at NAc A_{2A} adenosinergic receptors on the pronociceptive effect of REM sleep deprivation. A- Administration of A_{2A} agonist (CGS 21680 24 ng) into NAc core had no effect on the decrease in mechanical nociceptive threshold induced by REM sleep deprivation, but impaired the increase in mechanical nociceptive threshold during sleep rebound (repeated measures ANOVA, with one within-subjects factor (time) and two between-subjects factors (sleep condition and treatment). The symbol "*" indicates a mechanical nociceptive threshold significantly lower than that of the control groups within the same time point and the symbol "+"

indicates a mechanical nociceptive threshold significantly lower than that of REM sleep deprived rats receiving vehicle within the same time point (Tukey's post hoc test, $p < 0.05$). Arrows indicate when CGS 21680 was injected.

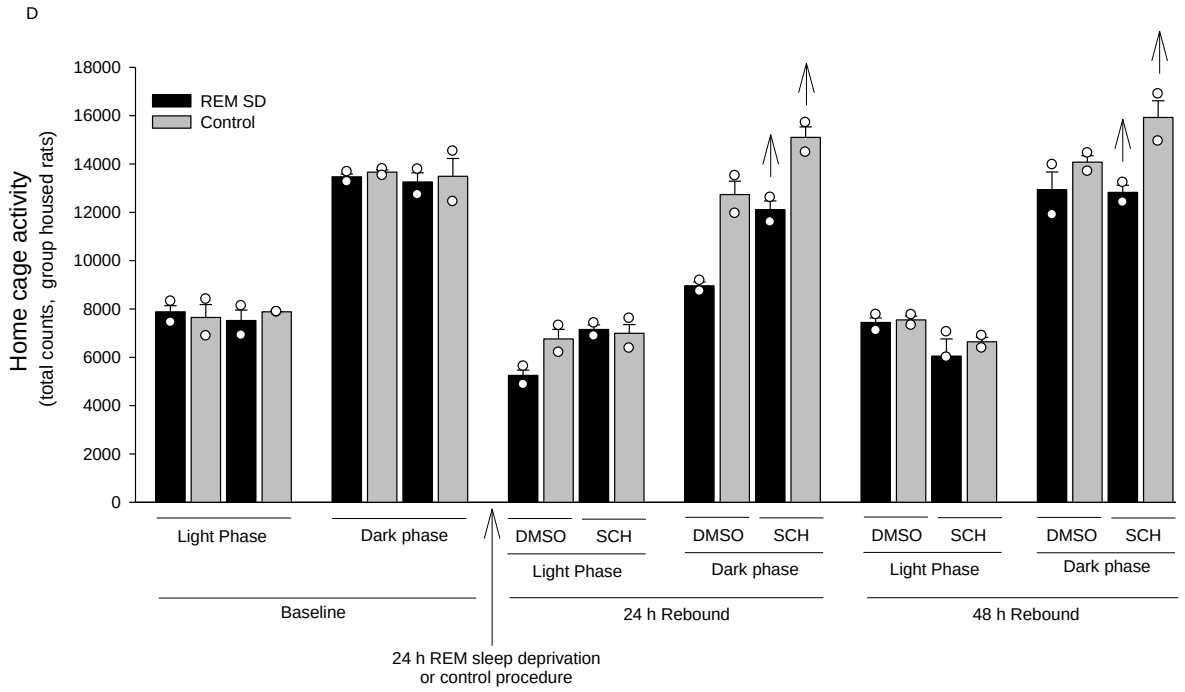


B- Qualitative assessment of home cage activity in group housed rats (four rats per cage, two cages per group) was compatible with decreased home cage activity during the light and dark phase in sleep deprived and control rats treated with the A_{2A} agonist at NAc core (the circles indicate individual values and the arrow the suggested alteration).



C- Previous administration of A_{2A} antagonist (SCH 58261 7 ng) into NAc core prevented the decrease in mechanical nociceptive threshold induced by REM sleep deprivation (repeated measures ANOVA,

with one within-subjects factor (time) and two between-subjects factors (sleep condition and treatment). The symbol "*" indicates a mechanical nociceptive threshold significantly lower than that of the control group within the same time point (Tukey's post hoc test, $p < 0.05$). Arrows indicate when SCH 58261 was injected.



D- Qualitative assessment of home cage activity in group housed rats (four rats per cage, two cages per group) was compatible with increased home cage activity during the dark phase in sleep deprived and control rats treated with the A_{2A} antagonist at NAc core (the circles indicate individual values and the arrow the suggested alteration).

The role of NAc dopamine D_2 receptors on the pronociceptive effect of REM sleep deprivation and in its progressive reversion during sleep rebound

The administration of the dopamine D_2 receptor agonist piribedil into the NAc core prevented the pronociceptive effect of REM SD (Figure 6 A repeated-measures ANOVA – sleep condition: $F(1, 19) = 1372.30$, $p < 0.0001$; treatment: $F(1, 19) = 2116.20$, $p < 0.0001$; sleep condition x treatment: $F(1, 19) = 1459.50$, $p < 0.0001$; sleep condition x treatment x time: $F(7, 133) = 220.73$, $p < 0.0001$. Post hoc analysis using Tukey's test indicated that REM SD failed to induce a pronociceptive effect in Piribedil treated animals $p > 0.05$), suggesting that decreased activity at NAc dopamine D_2 receptors contributes to the pronociceptive effect of REM SD.

Qualitative assessment of the home cage activity (Figure 6 B) suggests that the dopamine D_2 receptor agonist increased activity in the first dark phase in both REM sleep deprived and control animals (indicated by↑).

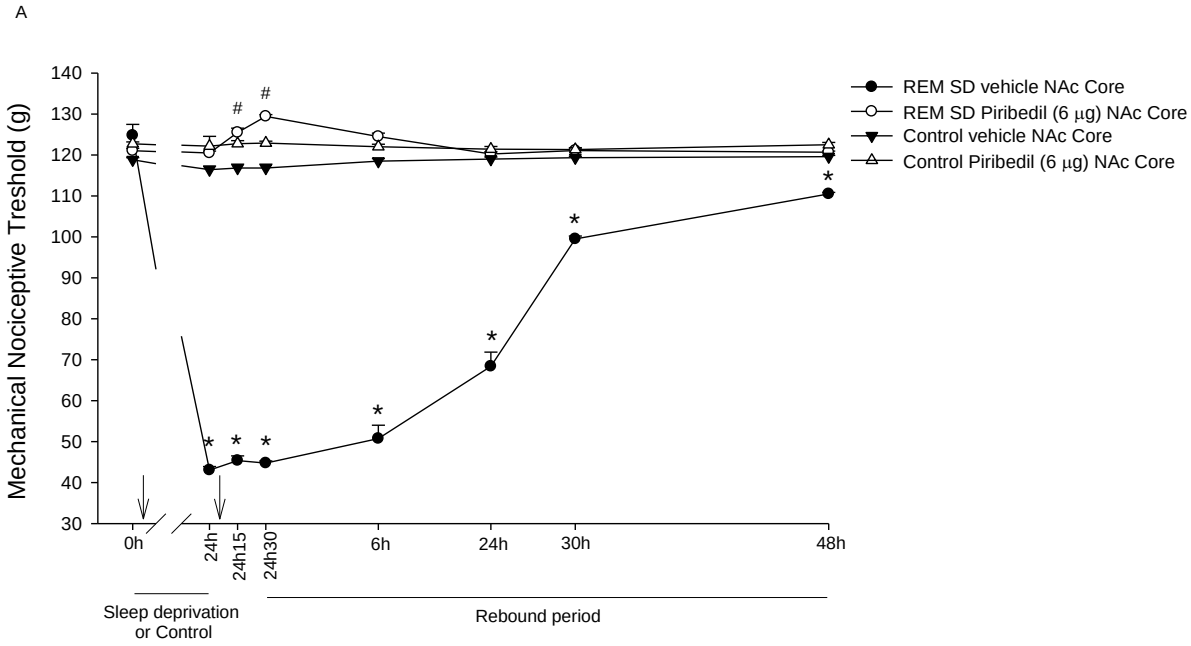
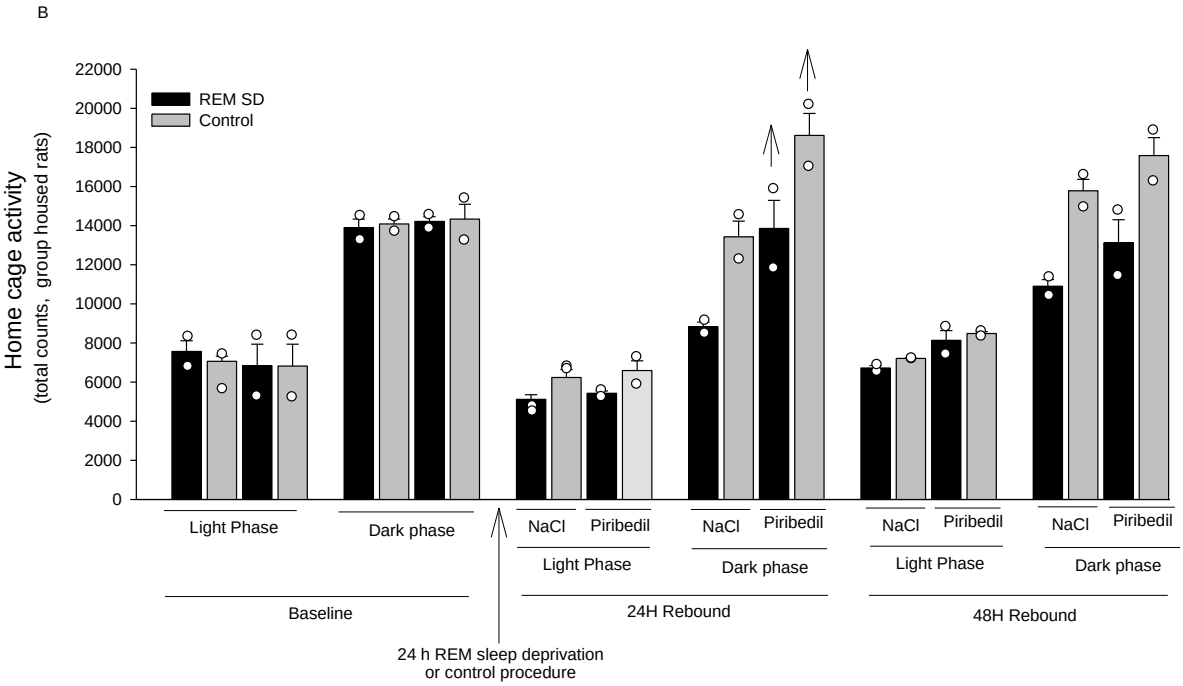


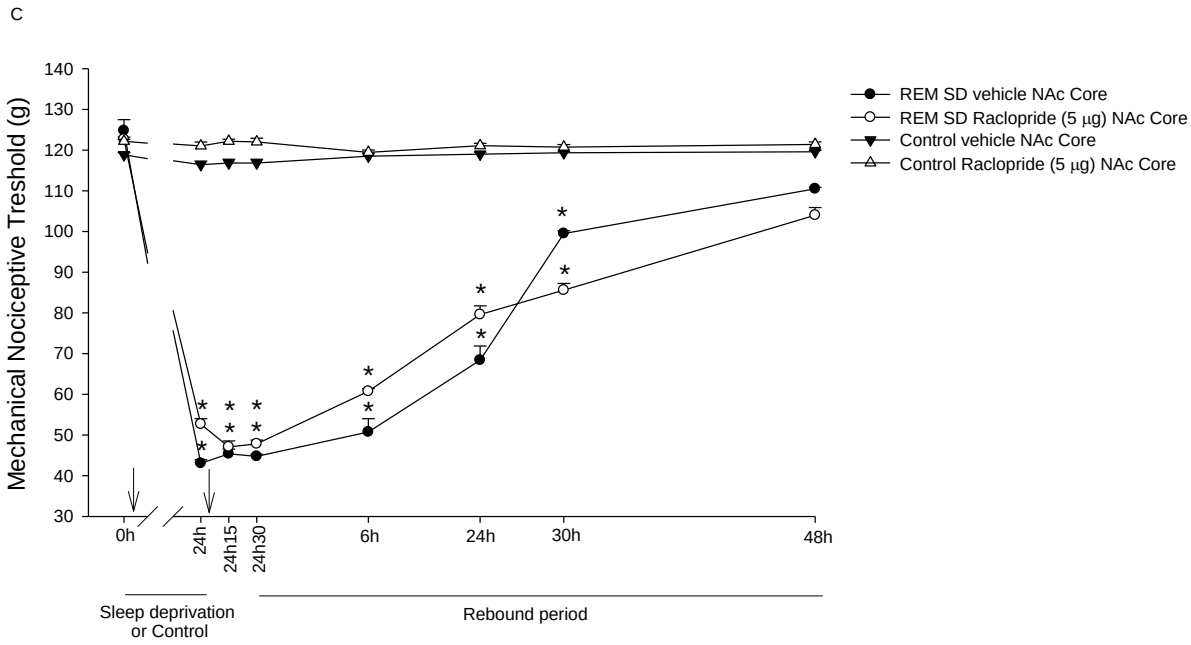
Figure 06: Effect of agonism and antagonism at NAc D_2 dopaminergic receptors on the pronociceptive effect of REM sleep deprivation. A- Administration of D_2 agonist (Piribedil 6 µg) into NAc core prevented the decrease in mechanical nociceptive threshold induced by REM sleep deprivation (repeated measures ANOVA, with one within-subjects factor (time) and two between-subjects factors (sleep condition and treatment). The symbol "*" indicates a mechanical nociceptive threshold significantly lower than that of the other groups within the same time point and the symbol "#" indicate a mechanical nociceptive threshold significantly greater than that of control group receiving vehicle within the same time point (Tukey's post hoc test, $p < 0.05$). Arrows indicate when piribedil was injected.



B- Qualitative assessment of home cage activity in group housed rats (four rats per cage, two cages per group) was compatible with increased home cage activity during the dark phase in sleep deprived and control rats treated with the D_2 agonist at NAc core (the circles indicate individual values and the arrow the suggested alteration).

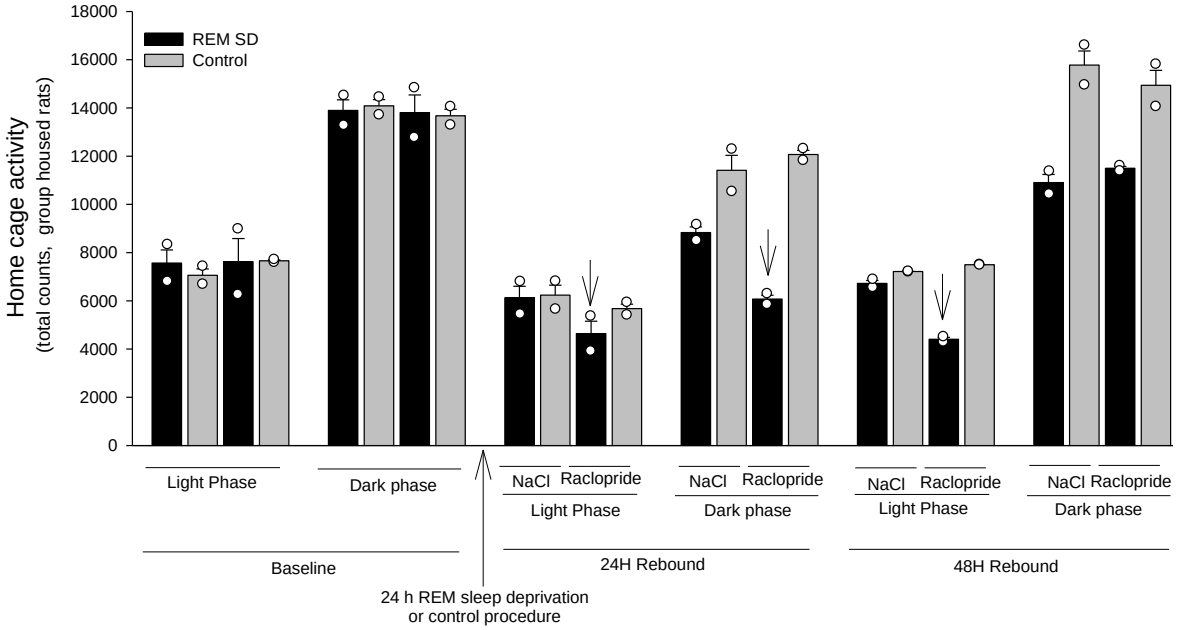
Administration of dopamine D_2 receptor antagonist raclopride into the NAc Core did not affect the pronociceptive effect induced by REM SD (Figure 6 C, repeated-measures ANOVA – sleep condition: $F(1, 20) = 120.69$, $p < 0.0001$; treatment (drug or vehicle): $F(1, 20) = 0.19$, $p = 0.663$; sleep condition x treatment: $F(1, 20) = 1.57$, $p = 0.224$; sleep condition x treatment x time: $F(7, 140) = 0.70$, $p = 0.669$. Post hoc analysis using Tukey's test indicated that raclopride treatment did not affect the pronociceptive effect REM SD $p < 0.05$).

Qualitative assessment of the home cage activity (Figure 6 D) suggests that the dopamine D_2 receptor antagonist decreased activity in the light and dark phases in REM sleep deprived animals (indicated by↓).



C- Administration of D_2 antagonist (Raclopride 5 µg) into NAc core had no effect on the decrease in mechanical nociceptive threshold induced by REM sleep deprivation (repeated measures ANOVA, with one within-subjects factor (time) and two between-subjects factors (sleep condition and treatment). The symbol "*" indicates a mechanical nociceptive threshold significantly lower than that of the other groups within the same time point (Tukey's post hoc test, $p < 0.05$). Arrows indicate when raclopride was injected.

D



D- Qualitative assessment of home cage activity in group housed rats (four rats per cage, two cages per group) was compatible with decreased home cage activity during the light and dark phase in sleep deprived rats treated with the D_2 antagonist at NAc core (the circles indicate individual values and the arrow the suggested alteration).

c-Fos expression

c-Fos expression did not significantly change after 24 h REM SD or during sleep rebound either into NAc core (Figure 7, One Way ANOVA, $F(1, 7) = 0.07$, $p = 0.786$) or shell (Figure 07, One Way ANOVA, $F(1, 7) = 1.88$, $p = 0.212$).

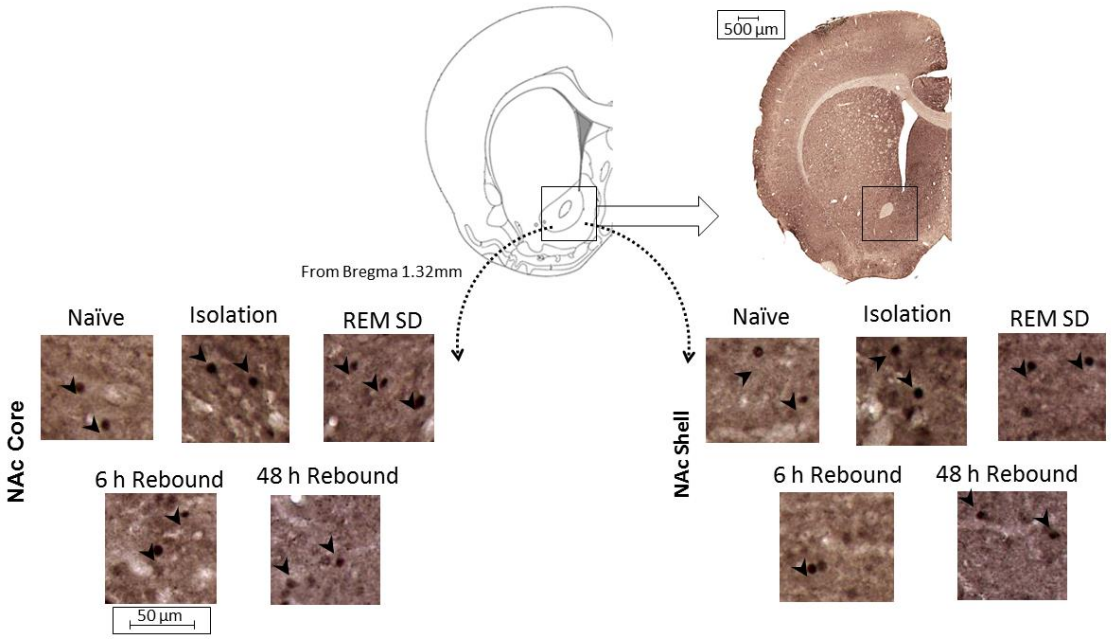


Figure 07 - c-Fos expression on NAc core and shell. Photomicrograph of representative sections for each group of c-Fos immunoreative (c-Fos-ir) cells in NAc core (left) and NAc shell (right). Arrows indicate the c-Fos-ir neurons within NAc core and shell. Diagrammatic representation on cross-sections from the atlas of Paxinos and Watson (2007). The numbers below the brain diagrams represent the atlas frontal coordinates in millimeters posterior to bregma.

Spontaneous Locomotion in a novel environment

REM SD for 24 h significantly increased spontaneous locomotor behavior in the open field test (Table 1, t test $p = 0.034$).

The excitotoxic lesion induced by previous administration of NMDA either into the NAc core (Table 1, two-way ANOVA – sleep condition: $F(1, 14) = 7.08$, $p = 0.018$; treatment (NMDA or sham lesion): $F(1, 19) = 2.55$, $p = 0.132$; sleep condition x treatment: $F(1, 19) = 1.98$, $p = 0.180$) or into NAc shell (Table 1, two-way ANOVA – sleep condition: $F(1, 19) = 4.29$, $p = = 0.571$; treatment: $F(1, 19) = 0.83$, $p = 0.377$; sleep condition x treatment: $F(1, 19) = 0.32$, $p < 0.577$) had no effect per se in locomotor behavior (Post hoc analysis using Tukey's test indicated that REM SD increased locomotor behavior in both NMDA and sham lesioned animals $p < 0.05$).

SPONTANEOUS LOCOMOTOR ACTIVITY			
CONTROL ANIMALS		**REM SLEEP DEPRIVED ANIMALS**	
Drug Treatment	Squares crossed (mean + EPM)	Drug Treatment	Squares crossed (mean + EPM)
Naïve	25.69 ± 1.54	Naïve	30.54 ± 1.15*
NAc Core NMDA (µg)		NAc Core NMDA (µg)	
0	24.50 ± 2.19	0	31.00 ± 1.57*
5,5	25.50 ± 1.57	5,5	34.00 ± 3.14*
NAc Shell NMDA (µg)		NAc Shell NMDA (µg)	
0	24.50 ± 2.22	0	32.00 ± 2.27*
5,5	25.80 ± 2.27	5,5	36.00 ± 4.45*
NAc Core Qx-314 (%)		NAc Core Qx-314 (%)	
0	25.33 ± 2.57	0	32.37 ± 1.90*
2	33.00 ± 2.60#	2	35.50 ± 2.33
NAc Shell Qx-314 (%)		NAc Shell Qx-314 (%)	
0	23.16 ± 1.85	0	30.77 ± 1.51*
2	23.60 ± 2.03	2	36.60 ± 1.95*+
NAc Core CGS 21680 (ng)		NAc Core CGS 21680 (ng)	
0	25.33 ± 1.74	0	32.37 ± 1.90*
24	22.83 ± 1.62	24	20.50 ± 0.75+
NAc Core SCH 58261 (ng)		NAc Core SCH 58261 (ng)	
0	25.60 ± 2.05	0	30.00 ± 2.63
7	24.83 ± 1.87	7	31.20 ± 2.15*
NAc Core Piribedil (µg)		NAc Core Piribedil (µg)	
0	25.33 ± 2.54	0	32.37 ± 2.20*
6	33.80 ± 2.10#	6	37.40 ± 2.78
NAc Core Raclopride (µg)		NAc Core Raclopride (µg)	
0	25.33 ± 1.74	0	32.37 ± 1.61*
5	21.33 ± 0.99	5	25.16 ± 1.53+

Table 1 - Effect (mean ± S.E.M.) of experimental manipulation on spontaneous locomotion. The symbols indicate "*" indicates significant ($p < 0.05$) difference between REM SD and control animals; "+" indicates significant difference between REM Sleep Deprived animals that received vehicle or drug and "#" indicates significant difference between control animals that received vehicle or drug (t test or two-way ANOVA with sleep condition and treatment as factors, followed by Tukey's post hoc test).

The acute blockade of NAc core by the administration of Qx-314 increased locomotor activity in control animals (Table 1, two-way ANOVA – sleep condition: $F(1, 19) = 3.63$, $p = 0.071$; treatment (Qx-314 or vehicle): $F(1, 19) = 4.64$, $p = 0.044$; sleep condition x treatment: $F(1, 19) = 0.82$, $p = 0.375$, Tukey's test $p = 0.042$). At NAc shell, Qx-314 increased locomotor activity in REM sleep deprived animals (Table 1, two-way ANOVA – sleep condition: $F(1, 25) = 7.63$, $p = 0.015$; treatment (Qx-314 or vehicle): $F(1, 25) = 0.53$, $p = 0.471$; sleep condition x treatment: $F(1, 25) = 2.02$, $p = 0.167$, Tukey's test $p = 0.026$).

The administration of the adenosine A_{2A} receptor agonist CGS 21680 into the NAc core did not affect locomotor activity in control animals, but prevented the increase in locomotor activity induced by REM SD (Table 1, two-way ANOVA – sleep condition: $F(1, 20) = 1.40$, $p = 0.249$; treatment (CGS 21680 or vehicle): $F(1, 20) = 13.11$, $p = 0.001$; sleep condition x treatment: $F(1, 20) = 5.57$, $p = 0.028$, Tukey's test $p < 0.01$)

The administration of the adenosine A_{2A} receptor antagonist SCH 58261 into the NAc core have no effect per se in locomotor behavior (Table 1, two-way ANOVA – sleep condition: $F(1, 19) = 2.17$, $p = 0.15$; treatment (drug or vehicle): $F(1, 19) = 0.08$, $p = 0.776$; sleep condition x treatment: $F(1, 19) = 0.002$, $p = 0.960$, Tukey's test $p = 0.035$).

The administration of the dopamine D_2 receptor agonist piribedil into the NAc core increased locomotor activity in control animals (Table 1, two-way ANOVA – sleep condition: $F(1, 20) = 4.21$, $p = 0.053$; treatment: $F(1, 20) = 6.77$, $p = 0.017$; sleep condition x treatment: $F(1, 20) = 0.44$, $p = 0.514$, Tukey's test $p = 0.043$).

The administration of the dopamine D_2 receptor antagonist raclopride in the NAc core prevented the increase in locomotor activity induced by REM SD (Table 1, two-way ANOVA – sleep condition: $F(1, 20) = 120.69$, $p < 0.0001$; treatment (drug or vehicle): $F(1, 20) = 0.19$, $p = 0.663$; sleep condition x treatment: $F(1, 20) = 1.57$, $p = 0.224$, Tukey's test $p = 0.037$).

Discussion

The main findings from this study demonstrated that 24 hours of REM SD induces an intense and long lasting pronociceptive effect that progressively decreases with sleep rebound, but still remains significant 48 h later. NAc mediates the pronociceptive effect of REM SD, since this effect was prevented by its previous excitotoxic lesion and was reverted by its acute blockade. Increased activity at adenosine A_{2A} and decreased activity at dopamine D_2 receptors located at NAc play a major role in the pronociceptive effect of REM SD. This is supported by findings showing that the administration of an A_{2A} receptor antagonist or of a D_2 receptor agonist into the NAc blocked the pronociceptive effect of REM SD.

The ability of SD in animals (Onen et al., 2000, Wei et al., 2007, Skinner et al., 2011, Tomim et al., 2016) and humans (Morin et al., 1998, Smith et al., 2007, Paul-Savoie et al., 2012, Schuh-Hofer et al., 2013) as well as sleep disturbance in humans (Smith and Haythornthwaite, 2004, Taylor et al., 2007) to increase pain responses has been consistently demonstrated. The present study reinforced and extend these findings showing that just 24 h of REM SD induces an intense and surprisingly long-lasting pronociceptive effect (figure 1 A). Although the magnitude of such effect decreases in the course of sleep rebound period, it still remains significant 48 h after REM SD. Animal's home cage activity was significantly decreased in sleep deprived rats during the dark, but not light phases within sleep rebound period (figure 1 B). This finding is consistent with increased sleep pressure and, consequently, increased sleep time during the phase in which the animals are expected to be more active. Literature data about sleep recovery time needed to return pain sensitivity to the basal values are discrepant, which could result, in part, from different nociceptive models and periods of SD. Although the neediness of short (24 h) sleep recovery periods has been demonstrated (Onen et al., 2000), most of the studies agree that longer periods are needed (Nascimento et al., 2007, Damasceno et al., 2009), in general, longer than the SD period (Hicks et al., 1979, Kozachik et al., 2015). These data, together with those from the present study suggest that an extended sleep recovery period is needed to reinstall the normality in nociceptive mechanisms after a single episode of SD. In a translational perspective, these findings could highlight the complexity and intensity of pain processing changes in patients suffering from sleep

disturbances or chronic SD due to occupational reasons. Understanding the mechanisms underlying the powerful effects of sleep loss in pain processing is the only way to succeed in the management of chronic pain conditions in such patients.

The mechanisms by which SD increases pain are largely unknown. This study has a significant contribution in this field, since it showed that the pronociceptive effect of REM SD is mediated by NAc, a component of the mesolimbic dopaminergic system, with recognized role in the modulation of both pain (Gear and Levine, 2011, Tobaldini et al., 2014) and sleep-wake cycle (Lazarus et al., 2013). The previous (20 days before experiment) excitotoxic lesion by NMDA either at NAc core or shell prevented the pronociceptive effect of REM SD and tended to increase dark phase activity in sleep deprived rats (figure 02). Similarly, the acute blockade by Qx-314, a quaternary derivative of lidocaine, either at NAc core or shell reverted the pronociceptive effect of REM SD while the acute blockade of NAc core, but not shell, tended to increase dark phase activity both in control and in sleep deprived rats (figure 03). Since the effect of Qx-314 is expected to last only few minutes (Saade et al., 2010, Gear and Levine, 2011, Ghanbarian and Motamedi, 2013), these last findings suggest that the transitory blockade of NAc activity is sufficient to largely decrease the pronociceptive effect of REM SD. Taken together, the findings from NAc lesion and acute blockade demonstrated that activity at either core or shell regions of NAc is essential to the induction and maintenance of the pronociceptive effect of REM SD. To our knowledge, this is the first evidence showing that SD increases pain by activating NAc dependent mechanisms. These findings are in line with those from a previous study showing that efferent activity from NAc facilitates nociception (Gear and Levine, 2011). Therefore, it is reasonable to suppose that SD increases pain by increasing efferent activity from NAc.

Recently, it was suggested that efferent activity from NAc, which is predominantly GABAergic, induces sleep (Qiu et al., 2010, Qiu et al., 2012) by inhibiting wake-promoting nuclei in the brainstem and hypothalamus (Lazarus et al., 2013). The NAc GABAergic efferent neurons express both D_2 and A_{2A} receptors, and their activity is regulated by a balance between dopamine acting on inhibitory D_2 receptors to induce wakefulness and adenosine acting on excitatory A_{2A} receptors to induce sleep (Lazarus et al., 2013). For example, activation of A_{2A} receptors at NAc induces sleep (Satoh et al., 1999), while their selective deletion at NAc prevents caffeine-induced wakefulness (Lazarus et al., 2011). Complementarily, when

administered at NAc, D_2 agonists decrease, while antagonists increase sleep (Barik and de Beaurepaire, 2005). Our findings obtained by measuring home cage activity in individually housed rats are in agreement with these previous one. The intra-accumbal administration of an A_{2A} receptor agonist decreased dark phase activity while an antagonist increased activity either in light or dark phases, and a D_2 agonist increases light phase activity while an antagonist decreased dark phase activity (figure 04). These findings are compatible with the well-established idea that NAc A_{2A} receptors increase and D_2 receptors decrease sleep and showed that the doses of agonists and antagonists used in this study are effective to induce their expected effects on sleep.

One of the major contributions of this study was to demonstrate that in addition to their role in sleep-wake cycle, A_{2A} and D_2 receptors located at NAc have a major role in the effect of sleep loss in pain sensitivity. The A_{2A} receptor antagonist prevented the pronociceptive effect of REM SD, while the agonist impaired its reversion during sleep rebound period (Figure 05 A). These findings suggest that SD could increase pain by increasing adenosinergic activity at NAc A_{2A} receptors. This suggestion perfectly fits with well-known role of adenosine as a mediator of sleepiness after prolonged wakefulness (Porkka-Heiskanen and Kalinchuk, 2011). In fact, a bundle of literature data clearly demonstrates that extracellular levels of adenosine increase in the basal forebrain and cortex of animals and humans during prolonged wakefulness or SD (Porkka-Heiskanen et al., 1997, Porkka-Heiskanen et al., 2000, McKenna et al., 2007, Rae et al., 2009). In clear agreement with literature (Huang et al., 2011, Lazarus et al., 2011), the qualitative assessment of home cage activity (Figure 05 B) suggests that animals receiving the A_{2A} receptor agonist slept more, while those receiving the antagonist slept less. Therefore, the A_{2A} receptor agonist impaired the reversion of the pronociceptive effect despite of apparently increasing sleep time during rebound period (increased need to sleep; increased pain), while the A_{2A} antagonist prevented the pronociceptive effect and apparently the need to sleep during the rebound period (decreased need to sleep; decreased pain). This indicate that the contribution of NAc A_{2A} receptors to the pronociceptive effect of REM SD appears to be directly linked to sleep pressure: the greater the need to sleep, the greater the pain sensitivity. In fact, although no previous studies have addressed the role of adenosine receptors at NAc in pain processing, findings from our control animals (not sleep deprived) indicate that neither the A_{2A} agonist nor the

antagonist affected nociceptive response, possibly because these animals were not under sleep pressure.

Findings from dopamine D_2 receptors complemented those from A_{2A} receptors, showing almost exact opposite roles of such receptors. The D_2 receptor agonist prevented the pronociceptive effect of REM SD, while the antagonist did not significantly change it (figure 06 A). These findings suggest that SD increases pain by decreasing activity at NAc D_2 dopaminergic receptors. In this scenario, the increased D_2 activity induce by the agonist would prevent, while its inhibition by the antagonist would not affect the pronociceptive effect, as our data have demonstrated. A decreased D_2 activity could result from a decreased dopamine release or/and a decreased D_2 receptor expression. A recent study performed both in humans and animals assessed this topic and conclude that SD decreases D_2 receptor expression in NAc (Volkow et al., 2012). This is noteworthy since adenosine through A_{2A} receptors decreases (Huang et al., 2013) while caffeine (an A_{2A} antagonist) increases (Volkow et al., 2015) D_2 receptor membrane availability in the striatum. In fact, it is almost intuitive to think that the increased sleep pressure in a sleep deprived subject is supposed to result from increased activity of sleep promoting mediators (adenosine) and decreased activity of wakefulness promoting mediators (dopamine). Again in clear agreement with literature (Qiu et al., 2012), qualitative analysis of home cage activity during sleep rebound period suggest that animals receiving the D_2 receptor agonist slept less , while those receiving the antagonist slept more. In parallel to findings from the A_{2A} agonist, the D_2 receptor antagonist apparently increased sleep time during rebound period, but this increase was not associated with a better reversion of the pronociceptive effect.

Despite of the essential role of NAc in regulating sleep and pain responses, its activity, indirectly estimated by c-Fos expression, was not significantly affected by REM SD (figure 7). No previous studies have quantified c-fos expression at NAc in response to SD. However, in a first view, this finding may appear to be in contrast with the increased adenosinergic activity at A_{2A} receptors that is expected to activate NAc output neurons to promote sleepiness (Lazarus et al., 2013) and, as demonstrated here, to increase pain. An explanation for such finding may rely on the fact that animals are unable to sleep. Although the increased sleep pressure mediated by adenosine at A_{2A} receptors would initially increase NAc activity, and theoretically, c-fos expression, whenever the animal fall off the platform and

wakefulness is forced, NAc would supposedly be inhibited. This could explain why c-fos expression at NAc was not significantly changed at the end of 24h of REM SD. In fact, it was demonstrated that c-fos expression initially increases during SD in areas (other than NAc) involved in sleep control, and then progressively decreases during forced wakefulness (Pompeiano et al., 1995, Dos Santos et al., 2013), reaching the basal levels with 24h of SD (Pompeiano et al., 1995).

Spontaneous locomotor activity in a novel environment is a general parameter of animal's behavior regulated by the striatum and phasically influenced by sleep conditions. In contrast to home cage activity, supposed to reflect sleep-wake pattern (Satoh et al., 1999, Barik and de Beaurepaire, 2005, Qu et al., 2010, Huang et al., 2011, Qiu et al., 2012), it is sensitive to phasic changes in neural circuits involved in motor control. For example, while SD decreases home cage activity (increases sleep time during rebound period), it increases locomotor activity in the open field test (table 01), a result classically attributed to the immediate dopaminergic supersensitivity induced by REM (Andrade et al., 1987, Dos Santos et al., 2013). Considering the role of NAc in the open field performance (Moreau and Huber, 1999, Pardo et al., 2013, Sanguedo et al., 2014), pharmacological intra-accumbal treatments affected spontaneous locomotion in an expected way, in which NAc blockade as well as the D_2 agonist increased it while the A_{2A} agonist and the D_2 antagonist prevented the increase in locomotion in response to REM SD. Noteworthy, drugs that decreased motor activity did not increased paw withdrawal threshold and drugs that increased motor activity did not decreased paw withdrawal threshold. Therefore, the results obtained with the nociceptive mechanical paw withdrawal test were not influenced by changes in motor behavior.

The limited knowledge about the mechanisms by which sleep loss increases pain contrasts with the broad advances in the field of sleep and pain neurobiology of the last decades. Experimental observations in humans suggest that SD or fragmentation impairs endogenous pain modulation (Edwards et al., 2009, Tiede et al., 2010, Paul-Savoie et al., 2012). In this regard, a recent study from our group demonstrated that REM SD disrupts the most known endogenous pain modulatory mechanism, the PAG-RVM descending system (Tomim et al., 2016). According to this study, REM SD increases pain by decreasing descending pain inhibitory activity and by increasing descending pain facilitatory activity (Tomim et al., 2016). Since PAG receives direct projections from NAc neurons expressing A_{2A} receptors (Zhang

et al., 2013), it is possible that the increased A_{2A} activity at NAc, in response to SD, activates a direct pathway to decrease descending inhibition and increase descending facilitation at PAG. Further studies are needed to test this hypothesis.

In summary, this study demonstrated that the increased adenosinergic A_{2A} and the decreased dopaminergic D_2 activity at NAc in response to sleep pressure (Satoh et al., 1999, Barik and de Beaurepaire, 2005, Huang et al., 2011, Lazarus et al., 2011, Qiu et al., 2012, Lazarus et al., 2013) mediate the potent pronociceptive effect of REM SD. The understanding of the mechanisms by which sleep loss affects nociceptive processing to increase pain (Smith and Haythornthwaite, 2004, Smith et al., 2007, Taylor et al., 2007, Edwards et al., 2009, Tiede et al., 2010, Paul-Savoie et al., 2012, Schuh-Hofer et al., 2013) and predict its future development (Lyngberg et al., 2005, Mork and Nilsen, 2012, Kim et al., 2015) is essential to succeed in the complex management of pain in patients suffering from sleep disturbances. For example, such patients could be more responsive to analgesics combined with caffeine (an adenosine antagonist) and/or to dopaminergic drugs.

Acknowledgments

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5. DISCUSSÃO

Este estudo demonstrou que 24 horas de PS REM induz um efeito pró-nociceptivo intenso e duradouro que diminui progressivamente com o rebote de sono, mas ainda permanece significativo 48 h depois. A atividade da fase escura durante o período de recuperação é significativamente diminuída em ratos privados de sono, o que é compatível com um tempo de sono aumentado após a PS REM. O NAc medeia o efeito pró-nociceptivo da PS REM, uma vez que este efeito foi evitado pela sua lesão excitotóxica prévia e foi revertido pelo seu bloqueio agudo. No entanto, o PS REM não alterou significativamente a expressão da proteína c-Fos no NAc. Os receptores A_{2A} de adenosina e os D_2 de dopamina no NAc desempenham um papel importante na atividade dos animais e no efeito pró-nociceptivo da privação de sono REM. A administração de um antagonista do receptor A_{2A} ou de um agonista do receptor D_2 no NAc aumentou a atividade e bloqueou o efeito pró-nociceptivo da PS REM, enquanto que a de um agonista do receptor A_{2A} ou de um antagonista do receptor D_2 diminuiu a atividade. O agonista do receptor A_{2A} também prejudicou a reversão do efeito pró-nociceptivo durante o rebote de sono.

A capacidade da privação de sono em animais (Onen et al., 2000, Wei et al., 2007, Skinner et al., 2011, Tomim et al., 2016) e humanos (Morin et al., 1998, Smith et al., 2007, Paul-Savoie et al., 2012, Schuh-Hofer et al., 2013), bem como distúrbios de sono em humanos (Smith and Haythornthwaite, 2004, Taylor et al., 2007) para aumentar as respostas a dor foi consistentemente demonstrada. Este estudo reforçou estas descobertas e mostrou que apenas 24 h de PS REM induz um intenso efeito pró-nociceptivo surpreendentemente duradouro (figura 1 A). Embora a magnitude desse efeito diminua no decorrer do período de recuperação de sono, ele permanece ainda significativo 48 h após a PS REM. A atividade da caixa do animal, medida durante 48 h após a PS REM, foi significativamente diminuída em ratos privados de sono durante a noite (fase escura), mas não nas fases claras (figura 1 B). Este achado é consistente com o aumento da pressão de sono e, consequentemente, do rebote de sono durante a fase em que se espera que os animais sejam mais ativos. Os dados da literatura sobre o tempo de rebote de sono necessário para a sensibilidade a dor retornar aos valores basais são discrepantes, o que pode resultar, em parte, de diferentes modelos nociceptivos e períodos de PS.

Embora a necessidade de períodos de curta duração de rebote de sono (24 h) tenha sido demonstrada (Onen et al., 2000), a maioria dos estudos concorda que são necessários períodos mais longos (Nascimento et al., 2007, Damasceno et al., 2009), em geral, mais longo do que o período de PS (Hicks et al., 1979, Kozachik et al., 2015). Estes dados, juntamente com os do presente estudo, sugerem que é necessário um período prolongado de rebote de sono para reinstalar a normalidade nas vias de processamento nociceptivo após um único episódio de PS. Em uma perspectiva translacional, esses achados poderiam evidenciar a complexidade e intensidade das alterações do processamento da dor em pacientes que sofrem de distúrbios de sono ou PS crônica por razões ocupacionais. Compreender os mecanismos subjacentes aos efeitos poderosos da perda de sono no processamento da dor é a única maneira de ter sucesso na gestão de condições de dor crônica em tais pacientes.

Os mecanismos pelos quais a PS aumenta a dor são em grande parte desconhecidos. Este estudo tem uma contribuição significativa neste campo, uma vez que mostrou que o efeito pró-nociceptivo da PS REM é mediado pelo NAc, um componente do sistema dopaminérgico mesolímbico, com papel reconhecido na modulação da dor (Gear and Levine, 2011, Tobaldini et al., 2014) e ciclo sono-vigília (Lazarus et al., 2013). A lesão excitotóxica prévia (20 dias antes do experimento) por NMDA, tanto no NAc core como no shell, impediu o efeito pró-nociceptivo da PS REM e tendeu a aumentar a atividade da fase escura em ratos privados de sono (figura 02). De forma semelhante, o bloqueio agudo por Qx-314, um derivado quaternário da lidocaína, tanto no NAc core quanto no shell, reverteu o efeito pró-nociceptivo da PS REM enquanto que o bloqueio agudo do NAc core, mas não do shell, tendeu a aumentar a atividade na fase escura tanto nos controles quanto nos ratos privados de sono (figura 03). Dado que o efeito do Qx-314 deve durar apenas alguns minutos (Saade et al., 2010, Gear and Levine, 2011, Ghanbarian and Motamedi, 2013), estas últimas conclusões sugerem que o bloqueio transitório da atividade do NAc é suficiente para, em grande parte, diminuir o efeito pró-nociceptivo da PS REM. Tomados em conjunto, os achados da lesão do NAc e bloqueio agudo demonstraram que a atividade do NAc é essencial para a indução e manutenção do efeito pró-nociceptivo da PS REM. Para nosso conhecimento, esta é a primeira evidência mostrando que a PS aumenta a dor ativando mecanismos dependentes do NAc. Estes resultados estão em linha com os de um estudo anterior mostrando que

a atividade eferente do NAc facilita a nocicepção (Gear and Levine, 2011). Portanto, é razoável supor que a PS aumenta a dor aumentando a atividade eferente do NAc.

Recentemente, sugeriu-se que a atividade eferente do NAc, que é predominantemente GABAérgica, induz o sono (Qiu et al., 2010, Qiu et al., 2012) inibindo núcleos promotores da vigília no tronco encefálico e no hipotálamo (Lazarus et al., 2013). Os neurônios eferentes GABAérgicos do NAc expressam os receptores D_2 e A_{2A}, sendo a sua atividade regulada por um equilíbrio entre a dopamina que atua sobre os receptores D_2 que são inibidores para induzir a vigília e a adenosina que atuam sobre os receptores A_{2A} que são excitatórios para induzir o sono (Lazarus et al., 2013). Por exemplo, a ativação dos receptores A_{2A} no NAc induz o sono (Satoh et al., 1999), enquanto a sua deleção seletiva no NAc evita a vigília induzida pela cafeína (Lazarus et al., 2011). Complementarmente, quando administrados no NAc, os agonistas D_2 diminuem, enquanto os antagonistas aumentam o sono (Barik and de Beaurepaire, 2005). Nossos achados, obtidos pela mensuração da atividade em ratos alojados individualmente, estão de acordo com os anteriores. A administração intra-accumbal de um agonista do receptor A_{2A} diminuiu a atividade da fase escura enquanto um antagonista aumentou a atividade quer na fase clara ou na escura, e um agonista D_2 aumenta a atividade da fase clara enquanto um antagonista diminui a atividade da fase escura (figura 04). Deste modo, os receptores A_{2A} no NAc aumentam e os receptores D_2 diminuem o sono.

Uma das principais contribuições deste estudo foi demonstrar que, além do seu papel no ciclo sono-vigília, os receptores A_{2A} e D_2 localizados no NAc têm um papel importante no efeito pró-nociceptivo da PS REM. O agonista do receptor A_{2A} não afetou o efeito pró-nociceptivo imediato da PS REM, mas prejudicou sua reversão durante o rebote de sono, enquanto que o antagonista do receptor A_{2A} impediu o efeito pró-nociceptivo (figura 5). Embora apenas qualitativos, nossos resultados com actimetria durante o período de rebote de sono sugerem que os animais que receberam o agonista do receptor A_{2A} dormiram mais (foram menos ativos), enquanto aqueles que receberam o antagonista dormiram menos (eram mais ativos) do que seus respectivos controles que receberam o veículo, o que está claramente de acordo com a literatura sobre o papel dos receptores A_{2A} no NAc na regulação de sono (Huang et al., 2011, Lazarus et al., 2011). Curiosamente, o agonista do receptor A_{2A} prejudicou a reversão do efeito pró-nociceptivo durante o período de rebote de sono apesar do tempo aparentemente crescente de sono.

Juntos, os resultados obtidos com a administração do agonista e antagonista do receptor A_{2A} no NAc sugerem que a PS REM poderia aumentar a dor aumentando a atividade da adenosina nos receptores A_{2A} no NAc. Essa sugestão se encaixa perfeitamente com o papel bem conhecido da adenosina como mediador da sonolência após vigília prolongada (Porkka-Heiskanen and Kalinchuk, 2011). De fato, um conjunto de dados bibliográficos demonstra claramente que os níveis extracelulares de adenosina aumentam no prosencéfalo basal e no córtex de animais e humanos durante a vigília prolongada ou PS (Porkka-Heiskanen et al., 1997, Porkka-Heiskanen et al., 2000, McKenna et al., 2007, Rae et al., 2009). Para nosso conhecimento, este é o primeiro estudo que investiga o papel dos receptores de adenosina no NAc no processamento da dor. É importante mencionar que nem o agonista A_{2A} nem o antagonista afetaram a resposta nociceptiva em animais controle (não privados de sono).

Os achados dos receptores D_2 de dopamina complementaram os de receptores A_{2A}, mostrando papéis opostos quase exatos de tais receptores. O agonista do receptor D_2 preveniu o efeito pró-nociceptivo da PS REM, enquanto que o antagonista não o alterou significativamente (figura 6). A análise qualitativa da atividade da caixa durante o período de recuperação de sono sugere que os animais que receberam o agonista do receptor D_2 dormiram menos (foram mais ativos), enquanto que aqueles que receberam o antagonista dormiram mais (eram menos ativos) do que seus respectivos homólogos recebendo veículo, o que está de acordo com a literatura sobre o papel dos receptores D_2 no NAc na regulação do sono (Qiu et al., 2012). Paralelamente aos achados do agonista A_{2A}, o antagonista do receptor D_2 aparentemente aumentou o tempo de sono durante o período de recuperação, mas este aumento não foi associado a uma melhor reversão do efeito pró-nociceptivo.

Juntos, os resultados obtidos com a administração do agonista e antagonista do receptor D_2 no NAc sugerem que a PS aumenta a dor diminuindo a atividade nos receptores dopaminérgicos D_2 no NAc. Nesse cenário, o aumento da atividade de D_2 pelo agonista evitaria, enquanto sua inibição pelo antagonista não afetaria o efeito pró-nociceptivo, exatamente como nossos dados demonstraram. Uma diminuição da atividade de D_2 poderia resultar de uma diminuição da liberação de dopamina e/ou de uma diminuição da expressão do receptor D_2. Um estudo recente realizado tanto em humanos como em animais avaliou este tópico e concluiu que a PS diminui a

expressão do receptor D_2 no NAc (Volkow et al., 2012). Isto é digno de nota, dado que a adenosina através dos receptores A_{2A} diminui (Huang et al., 2013) enquanto que a cafeína (um antagonista A_{2A}) aumenta (Kaasinen et al., 2004) a disponibilidade do receptor D_2 na membrana do estriado. De fato, é quase intuitivo pensar que o aumento da pressão de sono em um indivíduo privado de sono é suposto por resultar do aumento da atividade de mediadores promotores de sono (adenosina) e, diminuição da atividade de mediadores promotores da vigília (dopamina).

Apesar do papel essencial do NAc na regulação das respostas de sono e da dor, sua atividade, estimada indiretamente pela expressão de c-Fos, não foi significativamente afetada pela PS REM (figura 7). Nenhum estudo prévio quantificou a expressão de c-fos no NAc em resposta a PS. No entanto, numa primeira vista, esta descoberta pode parecer estar em contraste com a atividade adenosinérgica aumentada nos receptores A_{2A} que se espera ativar neurônios de saída do NAc para promover o sono (Lazarus et al., 2013) e, como aqui demonstrado, aumentar a dor. Uma explicação para tal descoberta pode depender do fato de que os animais são incapazes de dormir. Embora o aumento da pressão de sono mediada pela adenosina nos receptores A_{2A} inicialmente aumentaria a atividade do NAc e, teoricamente, a expressão de c-fos, sempre que o animal cair da plataforma e a vigília for forçada, o NAc seria supostamente inibido. Isto poderia explicar por que a expressão de c-fos no NAc não foi significativamente alterada ao final de 24h de PS REM. De fato, foi demonstrado anteriormente que a expressão de c-fos no NAc aumenta durante o sono REM (Sastre et al., 2000), quando se espera que a adenosina tenha ativado o NAc para promover o sono (Lazarus et al., 2013). Quando os animais são privados de sono, a expressão de c-fos aumenta inicialmente em diferentes regiões envolvidas no controle de sono e, em seguida, diminui progressivamente durante a vigília forçada (Pompeiano et al., 1995, Dos Santos et al., 2013), atingindo os níveis basais com 24 h de PS (Pompeiano et al., 1995), o que está de acordo com os presentes dados.

A atividade locomotora espontânea é um parâmetro geral do comportamento do animal regulado pelo estriado, diretamente influenciado pelas condições de sono e com impacto potencial em testes mecânicos nociceptivos. O aumento da atividade locomotora no teste de campo aberto (tabela 1) após a PS REM é um resultado esperado classicamente atribuído à supersensibilidade dopaminérgica induzida pela

PS REM (Andrade et al., 1987, Dos Santos et al., 2013). Em geral, os diferentes tratamentos intra-accumbal afetaram a locomoção espontânea de uma forma esperada, na qual a lesão/bloqueio do NAc bem como o antagonista A_{2A} e o agonista D_2 aumentaram, enquanto o agonista A_{2A} e o antagonista D_2 diminuíram. Esses achados se encaixam bem com os dados da atividade da actimetria e com a literatura (Moreau and Huber, 1999, Pardo et al., 2013, Sanguedo et al., 2014). É digno de nota, que os fármacos que diminuíram a atividade motora não aumentaram o limiar de retirada da pata e os fármacos que aumentaram a atividade motora não diminuíram o limiar de retirada da pata. Portanto, os resultados obtidos com o teste mecânico nociceptivo de retirada da pata não foram influenciados por alterações no comportamento motor.

O conhecimento limitado sobre os mecanismos pelos quais a perda de sono aumenta a dor contrasta com os amplos avanços no campo da neurobiologia de sono e da dor nas últimas décadas. Observações experimentais em seres humanos sugerem que a PS ou fragmentação prejudicam a modulação da dor endógena (Edwards et al., 2009, Tiede et al., 2010, Paul-Savoie et al., 2012). A este respeito, um estudo recente do nosso grupo demonstrou que a PS REM aumenta a transmissão da informação nociceptiva no mecanismo endógeno de modulação da dor mais conhecido, o sistema descendente PAG-RVM (Tomim et al., 2016). De acordo com este estudo, a PS REM aumenta a dor diminuindo a atividade inibitória descendente da dor e aumentando a atividade facilitadora descendente da dor (Tomim et al., 2016). Uma vez que a PAG recebe projeções diretas de neurônios do NAc que expressam receptores A_{2A} (Zhang et al., 2013), é possível que o aumento da atividade A_{2A} no NAc, em resposta a PS, ative uma via direta para diminuir a inibição descendente e aumentar a facilitação descendente na PAG. Mais estudos são necessários para testar esta hipótese.

Em resumo, este estudo demonstrou que o aumento da atividade A_{2A} adenosinérgica e a diminuição da atividade D_2 dopaminérgica no NAc em resposta à pressão de sono (Satoh et al., 1999, Barik and de Beaurepaire, 2005, Huang et al., 2011, Lazarus et al., 2011, Qiu et al., 2012, Lazarus et al., 2013) medeiam o poderoso efeito pró-nociceptivo da PS REM. A compreensão dos mecanismos pelos quais a perda de sono afeta o processamento nociceptivo para aumentar a dor (Smith and Haythornthwaite, 2004, Smith et al., 2007, Taylor et al., 2007, Edwards et al., 2009, Tiede et al., 2010, Paul-Savoie et al., 2012, Schuh-Hofer et al., 2013) e

prever seu desenvolvimento futuro (Lyngberg et al., 2005, Mork and Nilsen, 2012, Kim et al., 2015) é essencial para o tratamento complexo da dor em pacientes que sofrem de distúrbios de sono. Por exemplo, tais pacientes poderiam ser mais sensíveis a analgésicos combinados com cafeína (um antagonista de adenosina) e/ou fármacos dopaminérgicos.

6. CONCLUSÃO

Juntos os dados obtidos no presente trabalho sugerem que privação de sono REM aumenta a dor por aumentar a atividade adenosinérgica sobre receptores A_{2A} e diminuir a atividade dopaminérgica sobre receptores D_2 localizados no NAc. Entender os mecanismos pelos quais prejuízos no sono aumentam a dor é essencial para que se obtenha sucesso no complexo manejo da dor em pacientes que sofrem de distúrbios de sono. Por exemplo, baseado nos dados aqui apresentados pode-se esperar que esses pacientes se beneficiem especialmente de analgésicos combinados com cafeína ou de drogas dopaminérgicas.

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ANEXOS

Anexo 1 – Certificado da Comissão de Ética nº 923

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
Setor de Ciências Biológicas
Comissão de Ética no Uso de Animais
(CEUA)

UFPR
UNIVERSIDADE FEDERAL DO PARANÁ

Nº 923

CERTIFICADO

A Comissão de Ética no Uso de Animais do Setor de Ciências Biológicas da Universidade Federal do Paraná (CEUA/BIO – UFPR), instituída pela Resolução Nº 86/11 do Conselho de Ensino Pesquisa e Extensão (CEPE), de 22 de dezembro de 2011, **CERTIFICA** que os procedimentos utilizando animais no projeto de pesquisa abaixo especificado estão de acordo com a Diretriz Brasileira para o Cuidado e a Utilização de Animais para fins Científicos e Didáticos (DBCA) estabelecidas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e com as normas internacionais para a experimentação animal.

STATEMENT

The Ethics Committee for Animal Use from the Biological Sciences Section of the Federal University of Paraná (CEUA/BIO – UFPR), established by the Resolution Nº 86/11 of the Teaching Research and Extension Council (CEPE) on December 22nd 2011, **CERTIFIES** that the procedures using animals in the research project specified below are in agreement with the Brazilian Guidelines for Care and Use of Animals for Scientific and Teaching purposes established by the National Council for Control of Animal Experimentation (CONCEA) and with the international guidelines for animal experimentation.

PROCESSO/PROCESS: 23075.089951/2015-36

APROVADO/APPROVAL: 27/10/2015 – R.O. 11/2015

TÍTULO: Envolvimento do Núcleo Accumbens no efeito pró-nociceptivo induzido pela privação de sono REM em ratos.

TITLE: Involvement of Nucleus Accumbens in the pronociceptive effect induced by REM sleep deprivation in rats.

AUTORES/AUTHORS: Luana Fischer, Natalia Fantin Sardi, Glaucia Tobaldini, Vinicius augusto Guilhen.

DEPARTAMENTO/DEPARTMENT: Fisiologia

Profa. Dra. Ana Vitória Fischer da Silva
Coordenadora da CEUA

Anexo 2 – Certificado da Comissão de Ética nº 948

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
Setor de Ciências Biológicas
Comissão de Ética no Uso de Animais
(CEUA)

UFPR
UNIVERSIDADE FEDERAL DO PARANÁ

Nº 948

CERTIFICADO

A Comissão de Ética no Uso de Animais do Setor de Ciências Biológicas da Universidade Federal do Paraná (CEUA/BIO – UFPR), instituída pela Resolução Nº 86/11 do Conselho de Ensino Pesquisa e Extensão (CEPE), de 22 de dezembro de 2011, **CERTIFICA** que os procedimentos utilizando animais no projeto de pesquisa abaixo especificado estão de acordo com a Diretriz Brasileira para o Cuidado e a Utilização de Animais para fins Científicos e Didáticos (DBCA) estabelecidas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e com as normas internacionais para a experimentação animal.

STATEMENT

The Ethics Committee for Animal Use from the Biological Sciences Section of the Federal University of Paraná (CEUA/BIO – UFPR), established by the Resolution Nº 86/11 of the Teaching Research and Extension Council (CEPE) on December 22nd 2011, **CERTIFIES** that the procedures using animals in the research project specified below are in agreement with the Brazilian Guidelines for Care and Use of Animals for Scientific and Teaching purposes established by the National Council for Control of Animal Experimentation (CONCEA) and with the international guidelines for animal experimentation.

PROCESSO/PROCESS: 23075.116059/2016-16

APROVADO/APPROVAL: 01/03/2016 – R.O. 02/2016

TÍTULO: Envolvimento do Núcleo Accumbens no efeito pró-nociceptivo induzido pela privação de sono REM em ratos.

TITLE: Involvement of Nucleus Accumbens in the pronociceptive effect induced by REM sleep deprivation in rats.

AUTORES/AUTHORS: Luana Fischer, Natalia Fantin Sardi, Glaucia Tobaldini, Vinicius Augusto Guilhen.

DEPARTAMENTO/DEPARTMENT: Fisiologia

Profa. Dra. Ana Vitória Fischer da Silva
Coordenadora da CEUA